THE METABOLISM OF BROMINE IN MAMMALS

by

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I declare that except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and that, to the best of my knowledge and belief, the thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

Signed: .....J. Barta.....

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Adelaide, Joseph Barta.

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CONTENTS

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SUMMARY

The aim of the present investigation was to determine whether bromine has a physiological role in the mammalian body.

For this purpose the following investigations were carried out:
(1) Bromide levels and its possible binding to protein in the sera of human subjects and guinea pigs were examined. No protein bound bromine was found in either. The bromide ratios of red blood cells to serum and of the serum to C.S.F. were determined. The concentration varies between cells and serum in blood, but only to a limited extent, and is higher than in C.S.F. The blood concentration is influenced by diet. The bromide/chloride ratio in both serum and C.S.F. were also measured and was found to be different.

The serum bromide levels in a group of schizophrenic patients fell within the lower half of the normal range. This was shown to be due to the dietary factor.

(2) The bromide content in various tissues of man (including various regions of one human brain), of guinea pigs, of rabbits and of rats were examined by a chemical method. A radio-isotope method was also used but only in guinea pigs. The results showed that no tissue concentrates bromide preferentially, with the exception of the stomach (measured only in guinea pigs). No protein bound bromine was found in the pituitary.

The prolonged administration of varying amounts of bromide to guinea pigs showed that there was a progressive accumulation of bromide in all organs studied, the highest being in the serum.

The addition of fluoride to the drinking water of guinea pigs significantly decreased the bromide level in all tissues studied.

This suggests that fluoride can displace bromide in tissues.

(3) The effect of bromide and fluoride on thyroid function was investigated, and the results suggest that bromide may stimulate and fluoride depress the production of thyroid hormone.

(4) The rate of bromide excretion in humans was investigated using both radioisotopic and chemical techniques; the results showed that there was very little excretion during the first 24 hours (approximately 2%) and that the average biological half-life of bromide is approximately 6 days. Studies also indicated that chloride is excreted preferentially to bromide by the kidney. It was found that bromide is excreted at a reduced rate during the night. This diurnal rhythm of bromide excretion follows very closely that of chloride and sodium. There was no definite relationship between urinary volume and bromide excretion.

(5) Prolonged administration of bromide and fluoride to guinea pigs showed no significant effect on blood components, such as sodium, potassium, acetyl cholinesterase and alanine amino transferase (G.P.T.).

GENERAL INTRODUCTION

Bromide is widely distributed in soils, rock and especially water. It has been estimated that this element constitutes approximately 0.001 per cent of the earth's crust, and it occupies 25th place on the list of elements arranged in the relative order of their abundance. (1)

Since the elements of the halogen group are generally associated in nature it would be expected that they are absorbed together by plants and animals and should therefore be presented as normal constituents of living tissue. The presence of chloride, iodide and fluoride in most living organisms has been known for some time, while that of bromide has been in doubt. However it is now accepted that bromide is present in almost all biological fluids and tissues. In humans and animals, the bromide content depends upon the dietary intake. Winnek and Smith (1937) (2) found bromide to be present in most foodstuffs analysed, e.g.

Egg albumin	- 94.0 µg/g	White flour	- 5.2 - 7.9 µg/g
Milk powder	- 40.0 "	Alcoholic extract	
Whey powder	- 17.3 "	of wheat germ	- 8.7 - 8.9 "

Whether bromine is physiologically important to the body, or is simply present in the tissues and biological fluids as an accidental constituent ingested with all foods is still more or less unknown.

There appears to be some disagreement as to whether bromide has any nutritional value. Winnek and Smith (1937) (1) reported that

no significant difference was found in the general appearance, food intake, rate of growth or reproduction record between white rats on a synthetic diet low in bromide and those on a synthetic diet containing added bromide. Huff et al. (1956) (3), on the other hand, claimed that there is a nutritional requirement for bromide in rats, and Bosshardt et al. (1956) (4) reported an 8 - 10 per cent growth response in chicks on diets supplemented with a bromide source. However, the above evidence is not conclusive as there are several factors which might have influenced these findings. First of all it is a known fact that bromide crosses the placental barrier (see Chapter on Excretion of Bromide) and therefore it is found in the second generation originating from the mother; albumin is also known to contain bromide, and in any case it is very difficult to obtain a diet completely free of bromide.

Generally it could be stated that there is no evidence to date to show that bromide plays any physiological role in higher vertebrates, particularly mammals, however there have been reports suggesting that in some lower animals or plants, bromide may be somehow involved.

Burger and Ti Li Loo (1959) (5) suggested that certain marine animals possess an enzyme system which catalyses the biological halogenation of phenols. These authors showed that the uterus of the pregnant spiny dogfish, Squalus acanthias, an elasmobranch, can convert the dye phenol red (phenolsulfonphthalein) into a purplish - blue dye, bromophenol blue (3, 3', 5, 5' - tetrabromophenolsulfonphthalein).

Ti Li Loo et al. (1963) (6) found that the activity of this bromination was primarily in the uterine epithelium. The optimum pH

for bromination of 7.4 is close to the body pH of the fish (7). After 5 - 10 minutes of boiling, this epithelial tissue became completely inactive, thus indicating that an enzyme system was involved.

These studies were extended to include several other phthalein dyes, namely chlorophenol red, metacresol purple, and fluorescein; all of these were found to be brominated.

There are some other examples of biological bromination in nature. For instance, the bromination of phenol red was also observed by an enzyme system isolated from the mould Caldariomyces fumago at pH 2.8 (8); the natural occurrence of bromine compounds in marine animals such as the shellfish Murex brandaris and M. tranculus (9), and the coral fan Gorgonia verrucosa (10), indicates that enzymatic bromination may be of importance in their biogenesis.

It can be said that naturally occurring halogenated organic compounds, apart from thyroxin, are rare in animals. Therefore the examples cited are of great interest and argue for the existence in certain marine animals of an enzyme system which catalyses the bromination. According to the authors quoted biological halogenation of phenols may be analogous to the biogenesis of thyroxin, in the course of which tyrosine residues undergo iodination.

However, there seems to be no evidence as yet to show that bromide is involved in the physiology of mammals. It appears strange that while chlorine, fluorine and iodine all have definite physiological functions, there is no obvious function for bromine.

The aim of the present investigation was therefore to consider some aspects of the action of bromine in the biological systems of man

and guinea pigs and its relationship to other halogens.

This thesis is accordingly divided into five Chapters, each concerned with a different aspect of bromide investigation. Each individual Chapter begins with an appropriate historical background before the work of the present investigation is described. The latter then is subdivided into aims, methods, results and conclusions, in which there is an attempt to relate the present findings to those of previous workers.

The Chapters are as follows:

I. Bromide in the Blood. This deals with the bromide content in the blood of humans and guinea pigs, as well as bromide in human cerebrospinal fluid, its relationship to that in the blood and possible changes of these levels in disease. The relationship of bromide to the chloride content in these fluids is also considered.

II. Bromide in the Tissues. The analysis of various tissues for bromide content with the aim to determine whether any particular organ or organs concentrate bromide as, for example, iodide in the thyroid or fluoride in bone. Also the general mode of variation of bromide accumulation in tissues.

III. The Thyroid and Bromide. To study the possible effect of bromide on thyroid function, such as any interference with iodine metabolism.

IV. The Excretion of Bromide. A study of the rate and manner of bromide excretion in humans, and its relationship to other electrolytes, particularly chloride.

V. The Pharmacology and Toxicology of Bromide. Consideration is

given mainly to the effect of bromide administration to humans and animals in pharmacological doses.

The chemical method for the estimation of bromide is initially described.

HUNTER'S METHOD - MICRO-DETERMINATION OF BROMIDE IN BODY FLUIDS (11, 12)

This method was used for all chemical estimations of bromide in biological material during the present investigation. It is therefore described and discussed in detail. This micro-method for the estimation of bromide in the presence of chloride and in the absence of organic matter is based on the following reactions:

(1) Bromide is quantitatively oxidised to bromate in a suitable buffered solution by hypochlorite:

$$Br^- + 3ClO^- \longrightarrow BrO_3^- + 3\,Cl^-$$

(2) Bromate quantitatively gives six equivalents of bromine by reaction in acid solution with an excess of bromide:

$$BrO_3^- + 5\,Br^- + 6H^+ \longrightarrow 3\,Br_2 + 3H_2O$$

(3) Quantitative substitution of bromine in rosaniline decolourised by acid with the formation of a red solution (tetrabromorosaniline) which is measured colorimetrically.

The reaction (2) is carried out in the presence of rosaniline when the liberated bromine is found to react quantitatively.

Reagents (all of AR quality unless stated otherwise):

1. Sodium hypochlorite 1N in 0.1 N sodium hydroxide.

The method of preparation is as follows: the bromine-free chlorine, which is generated by gently heating a mixture of hydrochloric

acid and manganese dioxide, is washed by passing through water and then into cooled 1.1 N sodium hydroxide solution. From time to time 1 ml of this is removed into a small flask, 5 ml of 3% hydrogen peroxide is added to destroy the sodium hypochloride, and the solution is titrated with 0.1 N hydrochloric acid (using a suitable indicator) until the required 0.1 N NaOH concentration is obtained.

2. Sodium formate, 50% W/v.

3. Buffer solution at pH 6.35 prepared by mixing 10 volumes of 40% W/v sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), 7 volumes of 2N potassium hydroxide and 5 volumes of water.

4. Rosaniline solution. Prepared by dissolving 6 mg of rosaniline in 100 ml of 2 N sulphuric acid.

5. Bromide - molybdate mixture. By dissolving 0.14 g of potassium bromide and 3.0 g of ammonium molybdate in water and diluting to 100 ml.

6. Sulphuric acid, 14 N.

7. tert.-Butanol containing 5% v/v of absolute ethanol. The ethanol is added to prevent freezing at room temperature.

PROCEDURE.

(a) Preparation of the test solution.

Place in a nickel crucible 1 to 2 ml (or less) of biological fluids (serum, C.S.F., urine), add a few drops 2 N - NaOH and dry at 110°. Then ash at 600°C for 30 minutes in a muffle furnace. Remove the crucible and add 4 ml water. Stir with a glass rod and transfer the solution to a centrifuge tube and spin. Remove 3.5 ml of the

supernatant fluid into another test tube and add 1 ml buffer pH 6.35. Add 0.25 ml sodium hypochlorite solution, mix and heat for 10 min. in boiling water. Then add 0.25 ml sodium formate solution (to destroy the excess of hypochlorite), mix and heat for a further 5 minutes in boiling water. Remove, cool and add 0.2 ml water (the amount lost by evaporation). This is the test solution.

(b) Colour reaction.

In a test tube place 0.1 ml bromide - molybdate mixture, 0.5 ml rosaniline solution, 0.4 ml 14N-H_2SO_4 and 1 ml of the test solution. Mix and leave this reaction mixture for 3 minutes at 20 - 30°C, then add 2 ml tert.-butanol and 1 ml 14N - H_2SO_4.

Mix the solution and measure the optical density in a suitable absorptiometer at about 570 mμ. The action of tert.-butanol, a solvent, is necessary because the bromorosaniline is insoluble in the acid aqueous medium in which it is formed; tert.-butanol is convenient as this is mixable with the acid aqueous medium.

If the test solution contains much more than 5 μg Br^- as BrO_3^-, the colour of the final solution will be brownish owing to the destruction of the bromorosaniline formed by excess bromine. In such circumstances the amount of test solution should be reduced, but the volume should be kept constant by the addition of a corresponding amount of water before addition of the test solution. The amount of bromide present is readily calculable from the optical density reading, and the slope of the linear calibration curve for the absorptiometer used.

During the present investigation this method was first carried

out on NaBr and K Br solutions of varying concentrations from which a calibration graph was drawn up. This was done by dissolving varying amounts of K Br equivalent to 5, 10, 15, 20 and 25 µg Br^- per 4 ml of water, then taking off 3.5 of this and proceeding to make a test solution, from which 1 ml was taken for the colour reaction. The optical density thus obtained was plotted against the concentration to a maximum of 25 µg, after which concentration the calibration line ceased to be linear. That is, at this concentration (25 µg) each 1 ml of test solution contained 5 µg Br (as the total volume during the preparation of the test solution was 5 ml). In this way it was possible to read off the concentration up to 25 µg of the original biological material. Figure 1 shows this calibration graph.

After this, two human sera were estimated and the wave-length of maximum absorption of tetrabromorosaniline was checked. As can be seen from Figure 2, both sera showed that the maximum absorption was at 570 mµ.

A recovery experiment was also performed by adding known quantities of bromide (as NaBr) to the serum, mainly to test whether there is a significant loss of bromide associated with the destruction of organic matter during the ashing of serum. The tests were carried out in triplicate and an average taken. The results are shown below.

No.	Serum µg/ml	Added µg Br/ml	Expected µg Br/ml	Found µg Br/ml	Recovered %
1	9.3	3	12.3	12.0	97.6
2	9.0	5	14.0	14.0	100
3	5.0	5	10	9.5	95

No.	Serum µg/ml	Added µg Br/ml	Expected µg Br/ml	Found µg Br/ml	Recovered %
4	8.4	5	13.4	11.0	82.1
5	5.2	10	15.2	15.0	98.7
6	8.0	10	18.0	16.2	90
7	3.5	10	13.5	12.6	93.3
8	7.5	10	17.5	15.0	85.7
9	7.0	10	17.0	14.0	82.3
10	6.5	10	16.5	16.7	101.2
11	7.5	15	22.5	21.6	96
12	12.0	15	27.0	25.7	95.2

Hunter's method as described above, which was used for estimating bromide in biological fluids, was adapted in the present investigation to estimate the bromide content in various tissues, and proved to be quite successful. The main problem was to destroy most of the organic matter without a significant loss of bromide. Hunter suggested (12), as a means of destroying organic matter in the case of whole blood, to ash for 10 minutes in a furnace, cool and break down the charred mass with water, re-drying at 110°C, then re-igniting for 20 minutes. However, during the present investigation it was found that if dried tissue is slowly ashed at 200 - 300°C for approximately 30 minutes, and then the temperature is increased slowly to 600°C for another 30 minutes, this procedure destroys most organic material without any significant loss of bromide.

Hunter's method proved to be very useful for the estimation of relatively small quantities of bromide in biological material. Most of the tests were carried out in duplicate. A blank, which consisted of a few drops of 2 N NaOH in a crucible, which was then treated as other samples was included in every batch. The optical density of this was negligible in most cases. A control was also included in each batch; this consisted either of a solution of NaBr of known concentration, or a tested pooled serum.

If the bromide level was found to be too high for accurate bromide estimation the test was repeated with water dilution, and here it was found to be important that the water should be added before the test solution, or the results obtained were too high. The colour was very stable, as in 24 hours the drop in optical density was almost negligible, and in 48 hours only slight. Bromine-free hypochlorite was absolutely essential, and had to be prepared in the laboratory, as the commercial reagent was employed on two occasions and proved to be quite unsatisfactory as it showed a high optical density for blanks and tests.

Finally, it is important to note that only about 5 µg of bromide in one test can be correctly estimated without dilution (i.e., 25 µg/ specimen). If more bromide is present, the optical density decreases; e.g.

Sample A - no dilution - O.D. = .42

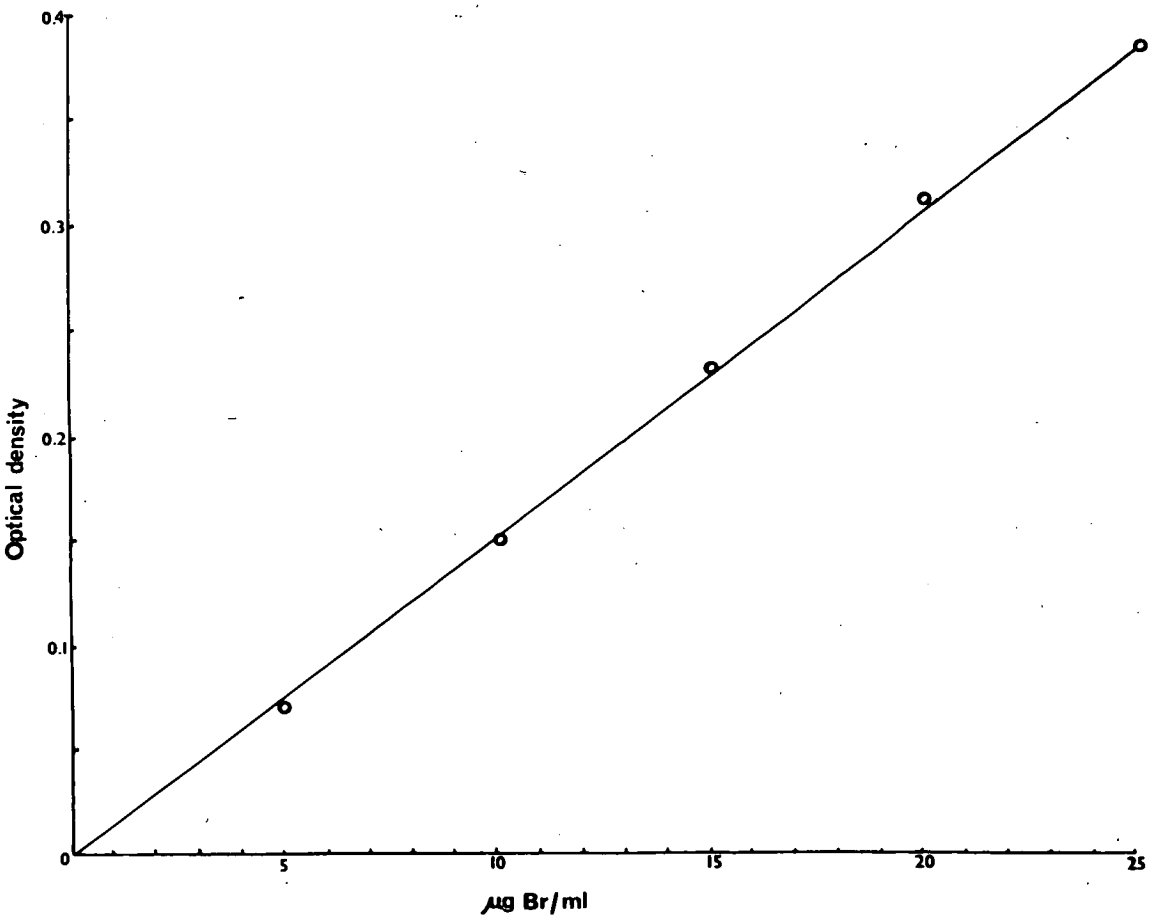
1 : 5 - O.D. = .45

1 : 10 - O.D. = .38

The reason for this has been stated above. Generally, providing one is

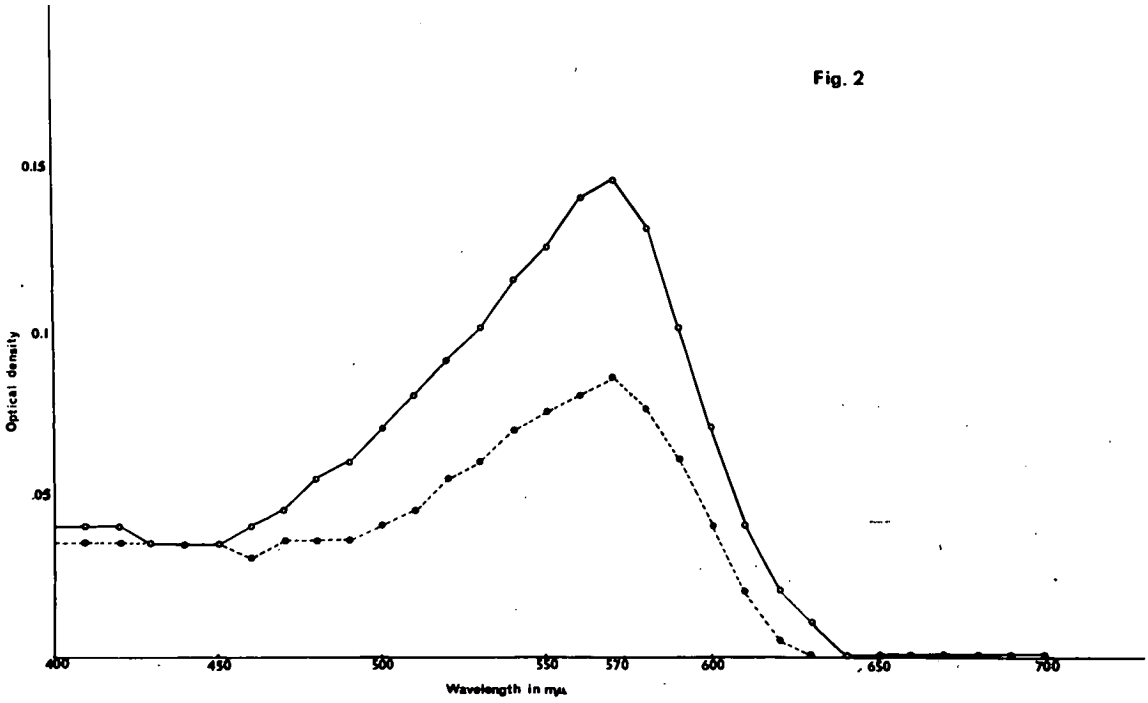
aware of its limitations, this method has proved to be very useful and reasonably reliable.

STANDARD GRAPH Fig. 1



MAXIMUM ABSORPTION OF TETRABROMOROSANILINE

Fig. 2



CHAPTER I

BROMIDE IN THE BLOOD

Damiens in 1921 (13) reported that cattle blood contains 0.52 mg bromide/100 g fresh weight, while that of dogs varies from 0.63 to 1.71 mg Br/100 ml. Bernhardt and Ucko in 1926 (14) found the blood bromide level for normal humans to be from 0.8 to 1.4 mg/100 ml and in the serum from 1.0 to 1.6 mg Br/100 ml. Similar blood bromide levels were found by Roman in 1929 (15). Ucko in 1936 (16) analysed the blood from normal human subjects and found the bromide content in approximately 100 samples to be between 0.15 and 0.35 mg/100 ml. Similar figures were found to apply to the blood of dogs. However, in a further 10 human subjects Ucko found that the range was from 0.6 to 3.0 mg/100 ml. He also found that the bromide/chloride ratio in the human blood was from 0.0005 - 0.0014, and claimed that about 1/5 of the total bromine is present in some organic complex.

Evans (1955) (17) claimed that the normal bromide level in humans was from 0.5 to 2.0 mg/100 ml of serum, while Hunter (1955) (12) found the bromide values in human whole blood to be 1.20 to 1.32, in blood cells 1.40 to 0.92 and in plasma 1.43 to 1.42 mg Br/100 ml.

Natelson et al. (1962) (18) reported the serum bromide level to range from 0.21 to 0.29 mg NaBr/100 ml, and Wikoff et al. (19) quoted a range from 0.1 to 3.3 mg NaBr/100 ml.

Some of the reasons for the differences in the results of the above authors will be dealt with in the "Discussion".

The Bromide Distribution Ratio between the Blood Cells and Serum.

(Q Br = Br concentration in red cells/Br concentration in serum.)

It has been claimed by several investigators that bromide is selectively concentrated in the red blood corpuscles. Hastings and Van Dyke in 1931 (20) claimed that when bromide is added to blood in vitro, it is distributed in such a way that the distribution ratio of bromide between cells and serum (Q Br) is about 10% higher than the corresponding chloride ratio (Q Cl), and they suggested therefore that the bromide replaces chloride from the blood cells. They further found that Q Br and Q Cl are lower in oxygenated blood than in reduced blood at the same pH, and increasing the pH of either oxygenated or reduced blood caused a decrease in both Q Br and Q Cl. In a subsequent paper (1931) (21) the same authors reported the results of their experiments in vivo. They claimed that when sodium bromide was administered to dogs by mouth, analysis of the blood revealed a much higher bromide and lower chloride concentration within the erythrocytes than would have been expected on the basis of the in vitro experiment. When a single dose of sodium bromide was given by mouth, the highest distribution ratio occurred 30 minutes after administration, then gradually decreased to the normal Q Br = 1.0 - 2.0 ratio. This ratio was constantly higher than Q Cl which was in the vicinity of 0.7. On the other hand, Q Br in vitro when NaBr was added, was never greater than unity. An attempt was made to determine the relative ease of diffusion of bromide from the cells of a bromide-fed dog into the serum of a normal dog and

conversely the ease of diffusion from the serum of a bromide fed dog into the cells of a normal dog. Blood samples were drawn from both animals and the bromide diffusion measured in vitro.

After 10 minutes of equilibration Q Br in the blood composed of bromide-containing cells and bromide-free serum was 1.45, and the corresponding Q Cl was 0.5. After $2\frac{1}{2}$ hours equilibration Q Br had fallen to 1.09 and Q Cl had risen to 0.685. The Q Br and Q Cl of the blood composed of bromide-free cells and bromide-containing serum were respectively 0.84 and 0.66 after 10 minutes and 0.82 and 0.72 after $2\frac{1}{2}$ hours equilibration. The latter ratios were approximately those obtained in blood to which bromide had been added in vitro, whereas the former ratios were much higher, and suggest a tendency on the part of cells from bromide-fed animals to retain their bromide.

Hastings et al. (1932) (22) extended the investigation of the bromide cell - serum ratio (Q Br) to other mammals. They concluded from their studies that after intravenous injection of sodium chloride and sodium bromide into the animals, the red blood corpuscles have a stronger affinity for bromide ions than for chloride ions. The Q Br is generally greater and usually to the same extent as Q Cl. The presence of bromide lowers the chloride ratios even further. These findings were criticised by Palmer and Clarke (1933) (23) on the basis that the work of Hastings et al. covered a relatively short period of time after a high dosage of bromide, and that insufficient time elapsed for equilibrium to be established. They maintained that, given adequate time for equilibration, the blood cells do not preferentially take up bromide.

Mason (1936) (24) who in turn criticised the method by which Palmer and Clarke obtained their results investigated the distribution of bromide and chloride in chronically bromide-intoxicated humans and dogs. His findings showed that Q Br tended to be slightly greater than Q Cl. The difference was small and variable but was of the order reported for the in vitro system by Hastings and Van Dyke (20) (i.e. Mason found that with time these ratios tended to fall to average values of 0.75 for bromide and 0.67 for chloride). These are, however, in contrast to Q Br = 2.0 found by Hastings and Van Dyke in vivo.

Ucko (1936) (16), on the other hand, failed to detect any selective concentration of bromide in the erythrocytes. He found that in humans the relative distribution of bromide between corpuscles and plasma was constant, whether it was present in normal or artificially increased amounts. Q Br was 0.33 and lower than was found for chloride. Weir and Hastings (1939) (25) reported that, after the administration of sodium bromide to dogs by mouth or by intravenous injection, Q Br was higher than the corresponding Q Cl (i.e. Q Br = 0.76 and Q Cl = 0.72). This therefore agreed with the observation made by Mason.

Smith et al. (1941) (26) compared the bromide, iodide and chloride distributions between human red blood cells and serum, and found the Q Cl to be between 0.8 to 1.2; Q Br in comparison was clearly greater than unity, i.e. from 1.24 to 1.46, and this was attained in 10 minutes and did not change even after three hours. Comparable results were obtained for Q I, which ranged from 1.20 to 1.36. These authors suggested that all three ions studied cross the red cell membrane at a

very rapid rate, since equilibrium is achieved in 10 minutes or less in each case. However, at equilibrium the cells contain a higher proportion of bromide and iodide than does the serum.

Mack and Shipley (1952) (27), using Br^{82} in rabbits and rats, found that the red cells contained approximately half as much bromide as plasma. Cole and Patrick (1958) (28) on the other hand reported that, following an injection of Br^{82} into bromide deficient rats, there was equal bromide distribution between the cells and plasma, with the ratio being maintained throughout the period of $1\frac{1}{2}$ to 96 hours. They claimed that there was initially a significantly higher concentration in the plasma.

Hunter et al. (1954) (29) reported that, in humans, equilibration of bromide between plasma and corpuscles is established within five minutes of the addition of bromide in vitro and within 30 minutes in vivo. They have also shown that bromide ratios between blood cells and plasma vary inversely as the pH, e.g.:

pH	8	7.2
Q Br in vitro	0.77	1.06
Q Br in vivo	0.66	0.96

Both of these findings are in accord with those of Hastings and Van Dyke (29). Hunter claimed that if the ratios are plotted against pH, they have about the same slope, with a shift of 0.3 pH unit causing a change of about 10% in the ratio. The chloride shift with pH has a similar magnitude (30). At normal blood pH the magnitude of the chloride ratio (Q Cl = Cl c/Cl p) is close to 0.70 for man (31).

As can be seen, there are conflicting reports on the distribution of bromide between the blood corpuscles and serum, ranging from the claim of significant concentration of bromide by blood cells, to slight concentration, to complete denial. Many factors are probably involved in these varying results, and some of these will be dealt with later in the "Discussion".

Blood and Cerebro-Spinal Fluid Bromide Ratio.

Stern and Ganlier (1921) (32) demonstrated the presence of bromide in the cerebro-spinal fluid (C.S.F.) of animals at intervals of $1\frac{1}{2}$, 3, and 16 hours after intravenous administration of large doses of bromide. Walter (1925, 1929) (33, 34) measured the bromide distribution ratio between serum and C.S.F. in humans, and concluded that the ratio which lay in the range of 2.90 to 3.30 was constant, and not influenced by time and the amount of bromide present. Mishkis et al. (1933) (35) reported this distribution ratio to be of the order of 1.5 to 2.0, and somewhat higher values from 2.4 to 2.8 were reported by Malamund et al. (1934) (36). Hunter et al. (1954) (29) found in 30 humans ratio values from 2.6 to 3.10. They also showed that the ratio is independent of the level of bromide in blood, thus supporting the earlier findings of Walter.

In considering these ratios there are several points stressed by numerous investigators which should be borne in mind:

(1) Methodology has to be considered as a factor in all cases where different authors appear to obtain divergent results.

(2) The time allowed for equilibration of bromide between serum

and spinal fluid following the administration of bromide is important. Walter (34) recommended an interval of 24 hours between the last oral dose of bromide and lumbar puncture. Wallace and Brodie (1940) (37) found, after intravenous injection of sodium bromide to a dog, the Br serum/Br C.S.F. ratio to be nearly constant after seven hours, and constant from 24 hours to 13 days. Hunter et al. (29) showed on human subjects that 24 hours should be the minimal interval between intravenous bromide dosage and lumbar puncture, while a considerably longer interval of at least 24 hours is generally accepted.

(3) Within the C.S.F., the bromide concentration decreases on ascending the neural axis. Thus Bau-Prussak (1927) (38) reported higher bromide values in the lumbar than in the cisternal fluids. Similar results were obtained by Masserman (1934) (39). Hunter et al. (29) found in "normal" humans that the bromide concentration of the ventricular fluid is about half that of the lumbar. The bromide level of cisternal fluid was found to be about midway between that of lumbar and ventricular fluid. It is clear, therefore, that values obtained will vary, depending on the position of puncture. Generally the lumbar C.S.F. is used for the estimation of serum/C.S.F. ratio.

(4) The effect of repeated lumbar punctures on the bromide level in the C.S.F. has also to be considered. Walter (34) mentioned this point and Fleischacker and Scheiderer (40) reported a fall of 4.5 to 9% in withdrawing successively three 5 ml samples of lumbar fluid. Hunter et al. (29) also observed a similar effect. It is therefore important that when more than one sample of fluid is taken, the first drawn fluid should be used for the bromide analysis.

It could be concluded that, although the bromide serum/C.S.F. ratio is influenced by the above four points, on the whole all investigators have found this ratio rather high in contrast with the values of 0.89 to 0.95 obtained for chloride (24, 41). Although it is now recognised that C.S.F. is secreted and is not in equilibrium with serum, it is nevertheless interesting that the bromide ratio differs so greatly from that for chloride.

Changes in Serum/C.S.F. Bromide Ratio.

It has been suggested by Taylor and Hunter (1957) (42) that the two to three times higher concentration of bromide in serum as compared with cerebrospinal fluid is due to the active exclusion and/or excretion of bromide from the C.S.F. There have been several reports, however, indicating that this serum/C.S.F. bromide ratio is changed in certain diseased states: thus Mason (24) studied the serum/C.S.F. bromide and chloride ratios in four bromide intoxicated hospital patients. He found that the serum/C.S.F. ratio for bromide of 1.2-1.4 was higher than that for chloride. According to Mason, NaCl therapy tends to increase this ratio and values as high as 4.5 were obtained during the period of salt therapy. Apparently in cases of prolonged intoxication, a larger proportion of bromide enters the spinal fluid than after a single dose or a brief period of bromide administration. The high ratios observed in the above mentioned patients during salt therapy point to the fact that bromide leaves the spinal fluid more rapidly than it leaves the serum.

The serum/C.S.F. ratio has been reported to be disturbed

during the course of tuberculous meningitis also. Walter (34) noted changes in the "normal" distribution of bromide between the serum and the C.S.F. in a human case of tuberculous meningitis. More recently Taylor et al. (from Hunter's Department) (1954) (43) and Smith et al. (44) reported that the blood-C.S.F. barrier to bromide is abolished, or profoundly depressed, during the course of tuberculous meningitis, and this barrier returns to normal when the infection is brought under control. Investigation on 33 patients with tuberculous meningitis revealed that in the majority of these cases the bromide serum/C.S.F. ratio was between 0.7 to 1.29 as compared with the normal of over 2.0. At the same time, the protein and cell content in the C.S.F. was increased. In other varieties of meningitis such a fall in the bromide serum/C.S.F. ratio proved to be exceptional, even though the changes in the amount of protein and the number of the cells were about the same as in tuberculous meningitis, and that on the whole, the blood-C.S.F. barrier for bromide was very well maintained (43).

Studies of the bromide ratio for ventricular (s/v), cisternal (s/c), and lumbar C.S.F. (s/l) show that the relationship $s/v > s/c > s/l$ is invariable. In active tuberculous meningitis all three ratios are depressed, but their relationship is never reversed.

It has been postulated by Hunter and his collaborators that the changes in the C.S.F. in tuberculous meningitis are the expression of spontaneous intrathecal tuberculin reactions, i.e. antibody-antigen response. This reaction may then inhibit active exclusion or excretion of bromide from the C.S.F. This hypothesis has been supported by the findings that the disturbance of the bromide serum/C.S.F. ratio in

tuberculous meningitis is identical with that of the experimental intrathecal tuberculin reaction. In these studies tuberculin has been introduced into the C.S.F. of experimental humans and animals with normal meninges. Tuberculin is a concentrated filtrate from the fluid medium on which the T.B. organism has been grown. It possesses antigenic properties but no infective property as it contains no living organisms. Injected tuberculin therefore will set up an antigen-antibody reaction without causing actual infection. This intrathecal tuberculin reaction is thus a true specific antigen antibody reaction which affects both the cellular and protein content of the C.S.F. This reaction had a great effect on the passage of bromide from blood to C.S.F. During the first phase of the reaction, the serum/C.S.F. bromide ratio falls to or below unity; in other words, the C.S.F. barrier to bromide is abolished. The ratio remains at this low level throughout the reaction, and then slowly begins to rise, but many weeks elapse before the barrier is completely re-established. The rise of the C.S.F. bromide level is often, but not invariably associated with a rise of the C.S.F. protein level. At the beginning of the reaction the serum/C.S.F. bromide ratio falls as the C.S.F. protein rises, but the C.S.F. protein level returns to normal before the serum/C.S.F. bromide ratio (43).

According to these investigators the serum/C.S.F. bromide ratio is a reliable indicator of the permeability of the blood-C.S.F. barrier to bromide, as is shown by the fact that it is independent of the absolute amounts of bromide present in the blood and C.S.F. As it is difficult to diagnose the early stages of tuberculous meningitis,

they have suggested that the alteration in the serum/C.S.F. bromide ratio could be useful in the routine examination of suspected cases.

PRESENT INVESTIGATION

AIMS.

1. To estimate the serum bromide level in "normal" human subjects in order to establish the mean and range of serum bromide level in the local population (South Australia).
2. To investigate whether there is any significant change in the serum bromide level in mental patients, particularly schizophrenics. This was done as the result of a claim of Zondek and Bier (1932-33) (45, 46) that the normal bromide content of the blood and C.S.F. was greatly diminished during the different stages of maniac-depressive psychoses. A second reason for this investigation was the known effect of bromide on the nervous system.
3. To establish the serum bromide range in normal guinea pigs. This was important in view of the experimental work performed on the guinea pig during the present investigation. Some guinea pig as well as human sera were tested for organically bound bromine.
4. To calculate the red blood cells/serum bromide ratio in humans and guinea pigs to find out the distribution of bromide between the blood cells and serum.
5. To measure the blood-cerebrospinal fluid bromide ratio in some patients with suspected neurological conditions.

METHODS AND RESULTS.

(1) Blood for serum bromide estimation was obtained from known

"normal" human subjects, and from hospital patients with normal Na, K and chloride levels. The bromide level was estimated by the chemical method in 96 human subjects. Most of the tests were carried out in duplicate. The results are shown in Table 1 and Figure 3. From the results obtained, the mean ($\frac{E x}{n}$) and standard deviation ($\frac{E(x - x)^2}{n}$) were calculated, and from these the range was established. The results of these calculations showed the mean as 9.3 and the standard deviation as 5.6, therefore the 95% range was $0 < 9.3 < 20.5$.

(2) The serum bromide level was estimated on 48 patients from a mental hospital, mainly schizophrenics. The results are shown below, and indicate that the serum bromide level in these patients mainly falls within the lower half of the normal range as established in experiment 1 above.

The results were as follows:

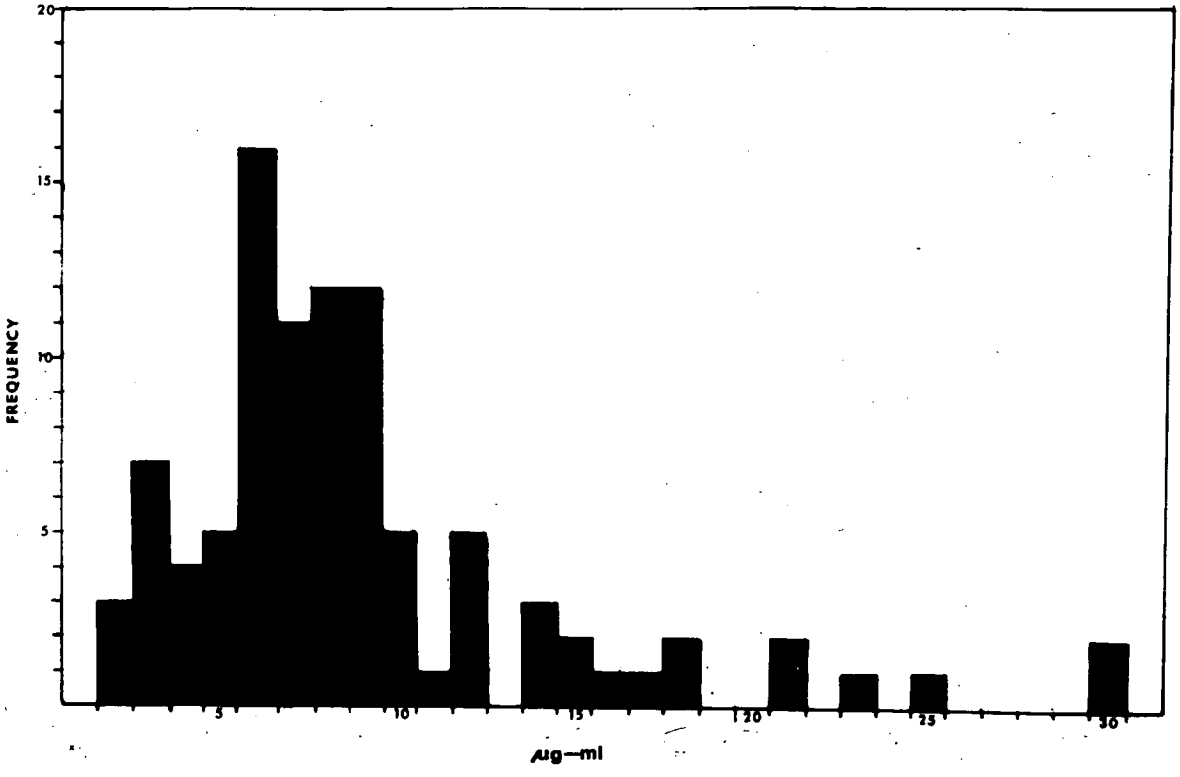
µg Br/ml serum	Frequency	µg Br/ml serum	Frequency
2.5	1	7.0	2
3.0	3	8.0	2
3.5	1	8.5	2
4.0	21	9.0	3
4.5	1	9.5	1
5.0	3	10.0	1
6.0	4	10.5	1
6.5	1	11.0	1

TABLE 1

DISTRIBUTION OF SERUM BROMIDE LEVEL IN 96 HUMANS

µg Br/ml	Frequency
2.0	3
3.0	3
3.5	3
4.0	2
4.5	2
5.0	5
6.0	10
6.5	6
7.0	7
7.5	4
8.0	4
8.5	5
9.0	8
9.5	4
10.0	4
10.5	1
11.0	1
12.0	3
12.5	2
14.0	3
15.0	2
16.0	1
17.5	1
18.0	2
21.0	2
23.0	2
25.0	1
30.0	2

DISTRIBUTION OF SERUM BROMIDE LEVEL IN 96 HUMANS Fig. 3



(3) The bromide level was also estimated by chemical means in the sera of 18 normal guinea pigs. The values obtained ranged from 4.5 to 11.0 µg/ml.

In order to determine whether there is any protein bound bromine in the serum, sera from 5 guinea pigs were precipitated with 10% trichloracetic acid, the precipitate was then washed 3 times with isotonic glucose solution (5%) and the bromide was estimated in this washed precipitate. The result in all cases proved to be negative, indicating that there is no protein bound bromine in guinea pig sera. This has been confirmed by measuring the radioactivity (Br^{82}) in the protein precipitate in two sera (after injecting Br^{82} into the animals) (see also Chapter on Tissues) which was practically negligible. Negative results were also obtained from the chemical bromide estimation of the serum precipitate from 10 human subjects.

(4) The red blood cells/serum bromide ratio was calculated on blood samples from 10 "normal" humans. This was done by taking whole blood (sequestrin used as anticoagulant) and measuring the blood cell volume (haematocrit). The individual blood specimens were divided into two parts - one of these was spun off and the plasma removed. The bromide content was estimated in a given volume of whole blood and plasma. (It has been found that there is no difference between plasma and serum bromide content.) The ratio cells/plasma was then calculated.

The following results were obtained.

No. of specimen	Cell/plasma ratio	No. of specimen	Cell/plasma ratio
1	1.11	3	0.80
2	0.66	4	0.98

No. of specimen	Cell/plasma ratio	No. of specimen	Cell/plasma ratio
5	1.01	8	0.53
6	0.85	9	1.08
7	0.76	10	1.18

A similar procedure was adopted in measuring the bromide content in whole blood and plasma of guinea pigs and then calculating the cell/plasma bromide ratio. In this case the estimation was performed on 3 animals, each of which had a high serum bromide content (i.e. the drinking water of these animals had contained 400 p.p.m. of bromide for a period of three months) as well as 6 normal guinea pigs.

The cell/plasma bromide ratios were as follows:

Animals with a high plasma bromide level

No.	Plasma bromide level µg/ml	Cell/plasma bromide ratio
1	424	0.14
2	324	0.70
3	312	0.74

Animals with a normal plasma bromide level

No.	Cell/plasma bromide ratio
1	1.70
2	1.73
3	0.54
4	1.62
5	0.93
6	2.07

(5) The cerebro-spinal fluid protein, chloride and bromide, as well as serum bromide contents were estimated on 4 human subjects with suspected neurological conditions. The serum/C.S.F. bromide ratio was then calculated. The results of these investigations are presented in Table 2. The bromide level in the C.S.F. of 11 human subjects was estimated in order to determine whether there is any relationship between the bromide level and the protein or chloride contents in these specimens of C.S.F. (see page 32).

DISCUSSION.

(1) A sample of 96 "normal" members of the population of South Australia had a 95% serum bromide level range of 0 to 20.5 µg/ml. As can be seen in Table 1 and Figure 3, the greatest frequency of sera samples had values between 5 and 10 µg Br/ml, i.e. 59.3% of the total specimens occurred in this 5-10 µg Br/ml range. The effect of varying dietary intake may play a large part in the spread of the serum bromide levels in the above sample of the population.

When the above results are compared with those obtained by some other authors (see Table 3) the range of the bromide level in human serum is seen to be narrower, except in the case of Evans (17). Whether this is actually so, however, depends on the number of specimens examined, and it is not always clear how many specimens were tested by these various workers. On the whole, the values obtained by these workers are not very different from those of the present investigation, with the exception of the results of Natelson et al. (18) whose figures

TABLE 2

INVESTIGATION OF SERUM/C.S.F. BROMIDE RATIO IN INDIVIDUAL HUMANS

Human Subject	A	B	C	D
Sex	Male	Female	Male	Male
Age in years	38	48	75	66
C.S.F.-Protein mg/100 ml (N = 10 - 40)	60	55	70	36
C.S.F.-Chloride m-equiv/litre (N = 120 - 130)	132	123	118	125
C.S.F.-Bromide µg/ml	3.1	5.2	2.5	2.5
Serum-Bromide µg/ml	9.5	3.0	4.0	4.5
Serum/C.S.F. Bromide Ratio	3.06	0.58	1.60	1.80
Diagnosis	Undiagnosed disease of nervous system	Depression, alcoholism, Epilepsy	Cerebral arterio-sclerosis, Epilepsy	Under investigation

TABLE 3

BLOOD AND SERUM BROMIDE LEVELS OBTAINED IN HUMANS AND ANIMALS BY VARIOUS AUTHORS

Author	Species	Blood (µg Br/ml)	Serum (µg Br/ml)
Damiens (1920)	Dogs	6.3 - 17.1	-
Bernhardt and Ucko (1926)	Humans	8.0 - 14.0	10.0 - 16.0
Roman (1929)	Humans	8.0 - 14.0	10.0 - 16.0
Ucko (1936)	Humans	1.5 - 3.5	-
Ucko (1936)	Dogs	1.5 - 3.5	-
Evans (1955)	Humans		5.0 - 20.0
Hunter (1955)	Humans	12.0 - 13.2	14.2 - 14.3
Natelson et al.(1962)	Humans		1.6 - 2.3
Present Investigation	Humans	5.2 - 22.5 (average 11.0)	0 - 20.5
" "	Guinea-pigs	4.0 - 18.8 (average 8.7)	4.5 - 11

are very low by comparison. Some of the probable reasons for the different serum bromide levels quoted by various authors are worthy of mention: the analytical method used; the dietary factors, and in addition it is not always clear whether the individual workers refer in their figures to bromide ions only, or to, for example, sodium bromide. In the present investigation figures always refer to bromide ions only. The bromide/chloride ratio in human serum was calculated to be on an average 0.0011 which is very similar to the figure obtained by Ucko (16).

(2) The estimation of serum bromide levels performed on schizophrenics was part of a wider investigation of different elements in the blood of these patients (47).

These serum bromide levels were found to be, on the whole, well within the lower half of the normal range. In order to determine whether this finding had any connection with the disease or whether some other factor such as diet was involved in the low bromide levels, blood was taken from three distinct groups from the same mental hospital. These groups each consisted of 6 human subjects: (a) known schizophrenics, (b) patients with other mental conditions, (c) staff members whose diets were similar to those of the patients. The results showed that the serum bromide levels in all three groups were similar, viz.:

(a) schizophrenics 4 - 6 µg Br/ml,

(b) non-schizophrenic patients 4 - 6.5 µg Br/ml,

(c) staff 3 - 6.5 µg Br/ml.

From the above figures the conclusion could be drawn that the dietary factor was the main cause for the relatively low serum bromide levels in all patients listed previously, and not, as was suggested by Zondek and Bier (45), due to the mental conditions of the patients.

(3) The range of the serum bromide level of guinea pigs (4.5 - 11.0 µg/ml) may not necessarily be much lower than that of humans, as only 18 guinea pigs were sampled, but all animals were living under the same conditions - dietary and otherwise. Therefore the dietary bromide intake was similar, and would have depended only upon the amount of food consumed by individual animals. This might explain the much narrower range as compared with humans. If, on the other hand, the intake of Br^- was increased the serum bromide level rose correspondingly (see Chapter on Tissues, Table 9). The negative results obtained by testing the protein precipitate from serum of humans and guinea pigs is contrary to the findings of Ucko (16) who claimed that about one-fifth of the total bromine content in the blood is present in protein-bound form.

(4) The red blood cells/serum bromide ratio of 10 human blood specimens was on the average 0.90 (range 0.53 - 1.18). This is in agreement with most other authors, with the exception of Smith et al. (26) and Ucko (16), whose results were higher and lower respectively (see Table 4).

The above ratio obtained in normal guinea pigs was higher than that of humans, with an average of 1.43 (range 0.5 - 2.07). Only

TABLE 4

BLOOD RED CELLS/SERUM BROMIDE RATIO FOUND BY DIFFERENT AUTHORS

Authors	Species	Bromide Ratio Cells/Serum
Hastings and Van Dyke (1931)	Dogs (administration of Br^-)	1.0 - 2.0
Mason (1936)	Humans and dogs (Br^- intoxicated)	0.75
Ueko (1936)	Humans	0.33
Weir and Hastings (1939)	Dogs	0.76
Smith et al. (1941)	Humans	1.24 - 1.46
Mack and Shipley (1952)	Rabbits and rats (using Br^{82})	0.5
Hunter (1954)	Humans	0.96
Cole and Patrick (1958)	Rats (using Br^{82})	1.0
Present investigation	Humans	0.53 - 1.18 (average 0.90)
" "	Guinea pigs (normal)	0.5 - 2.07 (average 1.43)
" "	Guinea pigs (Br^- treated)	0.14 - 0.74 (average 0.53)

one was well below unity, the other five were either near or well above unity. On the other hand, the guinea pigs with a high serum bromide level showed a much lower ratio (average 0.53), indicating that at a high blood bromide content a "saturation" point is reached, above which there is no increase of bromide content in the red blood cells.

Although this cell/serum bromide ratio in humans obtained by different workers varies, the majority of these workers found the ratio to be close to or above unity, with the exception of Ucko (16) and Mack and Shipley (27).

The above ratio in guinea pigs was higher than that found by most workers for other animals, with the exception of Hastings and Van Dyke (20) whose figures are slightly above those of the present investigation. Generally this bromide ratio for both humans and animals was higher than the corresponding chloride ratio of 0.67 - 0.72 found by numerous workers (20, 21, 24, 25).

There are several factors which could contribute to the varying results of the cell/serum bromide ratio obtained by different authors, but the most important of these is probably methodology. This includes not only the method of estimation of bromide, but also the general handling of specimens (e.g. pH change effect) and the red blood cells measurement, e.g. whether reliable haematocrits are performed, etc.

(5) The serum/C.S.F. bromide ratio obtained in 3 cases was from 1.6 to 3.0, and in a fourth 0.58. The three figures which are above unity agree with the findings of other workers for normal humans (see

Table 5). However, it is difficult to explain the ratio of 0.58, as other tests (e.g. C.S.F. protein content) and the final diagnosis did not suggest any significant change of permeability of the meninges. Unfortunately it was not possible to carry out the serum/C.S.F. Br^- ratio on a proven case of tuberculous meningitis to determine whether this ratio drops, and rises after recovery, as claimed by Hunter (43).

Although in the above experiment the protein content in the C.S.F. in three cases was above the normal range, there was no direct relationship between the bromide and protein levels in the C.S.F. This is confirmed by the results of measuring the relationship between the bromide and protein levels in 11 humans as seen in Table 6.

Finally, the bromide/chloride ratio in the C.S.F. was calculated and found to be 0.00032, as compared with a corresponding ratio in the serum of 0.0011.

TABLE 5

SERUM/C.S.F. BROMIDE RATIOS OBTAINED IN HUMANS BY VARIOUS AUTHORS

Author	Species	Serum/C.S.F. Bromide Ratio	Physical Condition
Walter (1925 and 1929)	Human	2.90 - 3.30	Normal
Mishkis (1932-33)	Human	1.5 - 2.0	Normal
Melamund et al. (1934)	Human	2.4 - 2.8	Normal
Hunter et al. (1954)	Human	2.6 - 3.1	Normal
Mason (1936)	Human	1.2 - 1.4	Bromide intoxicated
Taylor and Smith (1954)	Human	0.7 - 1.29	Tuberculous meningitis
Present investigation	Human	0.58 - 3.06	Various diseases of the nervous system

TABLE 6

THE RELATIONSHIP BETWEEN THE BROMIDE, CHLORIDE AND PROTEIN LEVELS IN THE CEREBRO-SPINAL FLUID

Human Subjects	Total Protein (mg/100 ml)	Chloride (m-equiv/l)	Bromide (µg/ml)
	(N. 10-40)	(N.120-130)	
1	40	120	3.4
2	50	133	1.5
3	145	118	18.5
4	28	133	1.8
5	8	128	1.8
6	96	-	2.8
7	34	124	13.5
8	55	128	2.8
9	72	128	32.0
10	100	128	4.0
11	50	129	2.5

CHAPTER II

BROMIDE IN THE TISSUES

Grange in 1851 (48) was probably the first to report the presence of bromide in animal tissues. He was followed by several other workers (49, 50, 51), but their results were contrasting and any of the positive results reported have only qualitative values as the methods used were not very accurate. Damiens in 1920 (52) gave an extensive survey of the methods used by all these authors, and criticised them from the viewpoint of later techniques. In a subsequent paper (1921) (13) he provided the first accurate quantitative bromide figures in human and animal organs. He found that the amount of bromide is fairly constant in these organs, and reported the following findings:

In the lungs, heart, liver, kidney and stomach of the chicken, the pheasant and the pigeon the bromide level ranged from 0.05 to 0.84 mg Br/100 g fresh weight. In the adrenals of cattle 0.13 and lung 0.42 mg Br/100 g fresh weight.

Values in various organs of dogs ranged from 0.37 to 1.71 mg Br/100 g fresh weight. In the human liver, lungs and kidneys the range was from 0.14 to 0.25 mg Br/100 g fresh weight. He was unable to find a bromide content corresponding to the iodine content in the thyroid. The blood bromide level in the above animals and humans examined was slightly higher than in most tissues (see the figures in Chapter I).

Damiens also claimed that the content of bromide was greatly increased in the lungs of humans who had died from poisoning with gases containing bromide (13). His findings were extended by Bernhardt and Ucko in 1926 (14) who found the following values in dogs:

Aorta, from 1.6 to 2.5; adrenals from 5.3 to 5.0; pituitary 12.5 mg Br/100 g fresh weight. In humans they found the highest values in the pituitary gland, viz. from 15 to 20 mg Br/100 g fresh weight. This was followed by the aorta, from 2.0 to 2.5, the adrenals from 1.4 to 1.8. In the liver, spleen and thyroid the range was from 0.6 to 1.4 mg Br/100 g fresh weight. They claimed, however, that the blood bromide level in humans is similar to that in the liver, spleen and thyroid, and considerably lower (about 20 times) than in the pituitary.

Picussen and Roman in 1929 (53) found the mean value of the bromide content of whole white mice to be 10.11 mg Br/100 g dry weight. Ucko in 1936 (16) reported the bromide content in some tissues of the cow as follows: thyroid 0.75 mg Br/100 g fresh weight, adrenals 0.62, pituitary 0.61, ovary 0.61.

Perlman et al. (1941) (54), three hours after an injection of Br^{82} into rats, found the following content of radio-active bromine in the various tissues, expressed as the percentage of the administered dose of Br^{82}/g: thyroid 2%, liver, kidney, salivary glands and the pituitary from 0.7 to 0.8%, adrenals from 0.31 to 0.37%, brain from 0.25 to 0.29%, whole blood about 1.4%. The latter contained the highest content of all tissues examined except the thyroid. They

obtained similar results in guinea-pigs, when, two hours after injection of Br^{82}, they recorded values in the thyroid from 0.52 to 0.88%, whereas in the liver, the adrenal gland, and muscle, the concentration did not exceed 0.32% of the administered dose of Br^{82}. Baumann et al. (55) also found that in rats all tissues studied except the thyroid had a lower concentration of Br^{82} than did the blood. The mean figures were: thyroid 0.99%, blood 0.67%, lung 0.42%, muscle 0.07% of the administered dose of Br^{82}.

Cole and Patrick (28) measured the Br^{82} uptake in bromide deficient rats and obtained the following results: intestine > kidney > spleen > gonads > thyroid = adrenals > pancreas > heart > liver > brain.

Mack and Shipley (27) claimed that 24 hours after injecting Br^{82} (1.0mCi/kg body weight) into rats and rabbits, the greatest activity can be found in the serum, then follows stomach > thyroid > gonads > kidney > spleen > pituitary > small intestine > adrenals > liver > large intestine > brain > muscle. They suggested that the relatively high values for stomach contents and stomach tissue might be explained on the basis of partial substitution of bromide for chloride in the gastric juice. I^{131} has been shown to behave similarly (56).

Gamble et al. (57) have shown that the concentration of bromide in the gastric content is as much as four to six times as great as would be expected from the chloride content. This has been supported by Davenport and Fisher (58) who claimed that gastric juice contains more bromide relative to chloride than does serum. The high

concentration of Br^{82} in the gastric content and mucosa has also been demonstrated in experimental animals by Söremark (59) and in man by Howe and Ekins (60) who showed that following the intravenous administration of Br^{82} an average of 20% of the administered dose was secreted into the stomach in 12 hours. The above results tend to confirm the early findings of Ucko (16) who claimed that in 10 "normal" humans, the content of bromide in the gastric juice of the fasting stomach is between 0.5 and 0.9 mg Br/100 ml and the Br/Cl ratio was between 0.0017 and 0.0055. The corresponding figures for blood were between 0.15 and 0.35 mg/100 ml and 0.0005 and 0.0014 respectively.

Numerous experiments have shown that with a bromide intake over a long period, changes in the bromide content occur in the organs and blood. Winnek and Smith in 1937 (1) experimented on two groups of white rats, each group having a different amount of bromide in the diet. The results showed that the blood and all tissues of the rats on a low bromide diet contained much less of this element than the blood and tissues of the animals receiving larger amounts of bromide. The tissues examined gave no indication that any one organ retained bromide more tenaciously than the others. The highest level was found in the kidney, closely followed by the spleen, with about half that amount in the liver which was equal to the brain level.

Nencki and Achoumow-Simanowski in 1894 (61) suggested that bromide displaces chloride in tissues. Several workers have shown since that when large doses of bromide are administered, the ratios of chloride to bromide are the same in the serum and in the tissues, with

the exception of the brain and the spinal fluid. This was confirmed by Weir in 1936 (62) who stated that bromide has no special affinity for any tissues and that the ratio bromide/chloride + bromide is constant in any animal for such diverse tissues as skeletal muscle, kidney, gut, skin, liver and blood.

The results of Wallace and Brondie in 1939 (63) were in agreement with Weir. They reported that in the tissues of dogs and cats bromide is distributed in the same ratio to chloride as in serum, presumably in proportion to the tissue extracellular fluid. In the brain, however, this ratio was considerably less, which suggested a barrier to its free passage from serum to brain, e.g. the wet tissue/serum ratio of bromide in the liver was 0.22, the brain cortex 0.12 and the cerebellum 0.22, whereas the corresponding figures for chloride were: liver 0.22, brain cortex 0.40 and cerebellum 0.52 respectively. The chloride concentration of the C.S.F. was found to be higher than that of the serum. They suggested that the fact that bromide and chloride are similarly distributed between the central nervous tissues and the C.S.F. involves ionic equilibrium between these tissues and the C.S.F. (63).

These results and the conclusions drawn from them were supported by Weir and Hastings in 1939 (25) who found that after the oral administration of sodium bromide to dogs, none of the tissues examined could be regarded as having a special preferential affinity for bromide rather than chloride. The degree to which chloride was replaced by bromide was essentially the same in all the tissues and plasma studied, except the brain and the C.S.F. They concluded that

there is (a) no special affinity for bromide in the tissues, and (b) bromide diffuses freely into and from tissues where chloride is present until equilibrium is established. The percentage replacement in the brain and the C.S.F. was lower, however, than in the blood and other tissues, and furthermore the replacement values in the brain and the C.S.F. agreed essentially with each other. They suggested therefore that the C.S.F. may be regarded as in equilibrium with the extracellular fluid of the brain tissue, at least in so far as halide ions are concerned, and in this sense, C.S.F. may accordingly be considered representative of the extracellular fluid of the brain.

Hellerstein et al. (1960) (64) examined the distribution of bromide in rat tissue at 6, 27 and 74 hour intervals after the intraperitoneal administration of sodium bromide. They analysed liver, muscle, skin and serum for bromide and chloride and found the ratio of chloride to bromide in the serum to be similar to the ratios of these ions in the tissues examined which confirmed the findings of the early workers (57, 63, 65, 66, 67).

There is evidence showing that when bromide is administered in pharmacological doses, it moves into the interstitial fluid in equilibrium with plasma in the same way as chloride, except in the brain and spinal fluid where bromide/chloride ratios are lower than in other organs (25, 63). In explaining the equilibrium between tissues in general and the serum, the assumption is made that the distribution of bromide in the tissues takes place in the extracellular water only; by analogy then, the corresponding extracellular water in the central

nervous tissues has the same relationship to the C.S.F. as that in other tissues has to serum (65). It is not surprising, therefore, that many workers (25, 66, 67, 68) recognised that bromide is not found in the brain in the same relation to chloride as it is found in the serum. It has been claimed (66) that the ratio of bromide of C.S.F. to that of serum is 0.75, while the corresponding ratio of chloride is 1.20. In the case of the brain, equilibrium is established with the halides in the C.S.F. rather than with those in the serum.

As has been mentioned, bromide and chloride are mainly distributed in the extracellular phase of tissue, and it therefore is necessary to define extracellular fluid: water makes up about 70% of the weight of the adult human body. This water is distributed throughout the body as intracellular and extracellular fluids. The intracellular fluids amount to about 50% of the body weight, while the extracellular fluids represent 20-22%. Extracellular volume (E.V.) has been defined as the volume of fluid which surrounds and bathes the cell (69). It can be visualised as including two major components: plasma water and interstitial water. Of the extracellular fluids, interstitial fluid amounts to about 15% of the body weight. The interstitial fluid may act as a fluid buffer between the plasma and the intracellular fluid for certain substances in certain tissues. Since plasma is subject to sudden variations in composition through absorption from the intestine, the imposition of interstitial fluid aids in maintaining the more constant composition of intracellular fluid than would otherwise be the case. With this arrangement, the kidneys have the

opportunity to compensate for changes in the plasma before they are seriously reflected upon the intracellular fluid.

The moiety of fluid in the central nervous tissue (including the cerebrospinal fluid), the eye, the gastrointestinal tract, the pericardial space, pleural space and joints has been called transcellular fluid, and this fluid is part of the extracellular phase; this is, however, only a very small percentage of the whole of the extracellular volume.

The extracellular fluid is very commonly divided in the literature into two main components, viz. the intravascular fluid (mainly plasma) and extravascular fluid (mainly interstitial fluid) and on this basis some authors speak of the ratio between tissues and plasma. This differing interpretation should be borne in mind when discussing the ratio between tissues/plasma or tissues/C.S.F.

Some ions such as potassium are found mainly in the intracellular fluid; on the other hand, many investigators have concluded from tissue analysis that chloride is predominantly, though not exclusively, extracellular (69, 70, 71, 72, 73, 74, 75).

Gotlove (76) infused insulin and sucrose into rats constantly for a prolonged period with subsequent analysis of the muscle for saccharide content to study the position of chloride in muscle tissue. These saccharides did not penetrate the tissue cells. He found that the saccharide and chloride spaces of the muscle were equal; in other words, no chloride was found within the muscle cell. Cheek et al. (77) extended this study to the viscera and found that the inulin space in

the viscera was almost equal to the chloride space. In the studies on rats (77) it was found that 90% of total body chloride was extracellular, while the majority of non-extracellular chloride was present in the red blood cells (6%) or in the visceral cells (1.8%). Edelman and Liebman (78) concluded that 12.4% of chloride is extracellular in man, therefore the correction of the chloride space in the whole body by a factor of 10% is normally considered to approximate the true extracellular volume.

This chloride space is frequently measured with substances that follow the distribution of chloride in the body very closely. Wallace and Brodie (63) compared tissue bromide/serum bromide and tissue chloride/serum chloride and concluded that the distribution of both halides was similar in all tissues examined except the brain. Similar results were obtained by Weir and Hastings (25) who found that there was agreement between the amounts of extracellular fluid per kilo of tissue calculated, on the one hand from chloride data and on the other from bromide data. Manery (75) showed that the volume of distribution of bromide in muscle closely approximates the chloride space of muscle. In general, it can be said that bromide and chloride distribute in a similar manner in all tissues (25, 57, 65, 66, 79) with the exception of the central nervous system, and possibly of the thyroid and the red blood cells (see Chapter I).

It is now generally accepted that administered bromide distributes itself principally, if not entirely, throughout the extracellular phases in tissue. Accordingly, bromide is frequently used

for the determination of total extracellular fluids. For this purpose the most useful form is radioactive bromide Br^{82}(33-37). It has certain advantages, viz. only small amounts are needed for injection, bromide is not metabolised in the body, and it is excreted slowly.

Fink and Cheek (80) reported that isotopes of chloride and bromide are of equal value in the determination of the corrected chloride spaces. A correction factor of 10% is applied to the bromide or chloride space to allow for intracellular chloride and bromide. In adult-life the corrected bromide space-water is equal to about 20-22% of body weight (65).

Considering that bromide is mainly distributed in extracellular fluid, it can be concluded that in the event that the tissue/plasma ratio of bromide for any particular tissue exceeds unity, conclusive evidence would be at hand that the cells of that tissue are able to concentrate bromide ions. If the cells of a particular tissue do not take up bromide ions, then the concentration of bromide ions in that tissue will depend on the amount of interstitial fluid and the vascularity of the tissue.

It has been shown that the majority of investigators in this field failed to produce evidence that any organ can selectively concentrate bromide ions. The thyroid may be an exception, as several workers found a higher concentration of bromide here than in the serum, but this appeared to depend on the iodine stores of the body (see Chapter on the Thyroid). Some of the early workers reported a high concentration of bromide in the pituitary. As mentioned above,

Bernhardt and Ucko in 1926 (14) found a very high concentration of bromide in the pituitaries of dogs. This has been supported by Zondek and Bier in 1932-33 (45, 46) who reported that, in dogs, the hypophyseal bromide diminished or disappeared during artificial sleep, whereas the bromide content of the mesencephalon increased. The authors suggested that the pituitary secreted a substance containing bromide which could act upon the cerebral centre producing sleep. The report suggested a physiological action of bromide; the work has, however, been repeated by other investigators and although some have substantiated it (81) others have been unable to confirm the results.

Dixon (1935) (82) and Ucko (1936) using newer analytical methods failed to find the existence of any preferential concentration of bromide in the hypophysis of ox, pig or man.

Perlman et al. (1941) (54) using radioactive bromide could not demonstrate a high concentration of radiobromide in the pituitaries of rats.

Mack and Shipley in 1952 (27) reported the results of their experiment on the distribution of Br^{82} in various tissues of rabbits and rats. They confirmed the findings of Perlman et al., and found that the tissue/serum ratio of bromide in the pituitary of rabbits was 0.28, while that of rats was 0.36. These figures contrast sharply with the ratio of 17-20 obtained by the early workers, and it would appear that the reliability of the early methods used is open to question.

PRESENT INVESTIGATION

AIMS.

(a) The Distribution of Bromide in the Various Tissues.

Although other workers have investigated the bromide content in the tissues of a number of animals, the analysis of guinea-pig tissues has been relatively neglected. Radio-isotopic and Hunter's chemical methods were used to estimate bromide distribution in guinea-pigs, and to determine whether any tissue selectively concentrates bromide.

Bromide levels in the tissues of the human, the rat and the rabbit were also studied and compared.

(b) The Pattern of Accumulation of Bromide.

It is known that when bromide is administered in pharmacological doses, it accumulates almost entirely in the tissue fluid spaces. This experimental work, however, is aimed at determining firstly whether all tissues take up bromide to the same extent, and secondly whether the tissue/serum ratio is less than unity in each individual tissue, as is the case in normal untreated animals. The results of these assays would indicate whether the prolonged administration of high doses of bromide alters the tissue/serum ratio of bromide.

(c) The Effect of Fluoride on the normal Bromide Level.

It has been stated in the previous pages that bromide can

replace chloride in the tissues and blood. The question then arises whether the normal bromide content in the tissues can be similarly replaced by other halides. This experiment was set up to ascertain whether fluoride ions affect the bromide level in the tissues and serum of guinea-pigs.

(d) Bromide in the Pituitary and Brain.

This experiment was carried out to investigate the claim of Zondek and Bier (45, 46) concerning the high bromide content in the pituitary and its possible physiological function. If their findings were correct, one would expect to find a relatively high concentration of bromide in the pituitary, most of it being organically bound, and possibly a relatively higher concentration of bromide in some parts of the brain. If the pituitary gland does in fact selectively accumulate bromide this would be of considerable interest, because of the implication that bromine may participate in the synthesis of pituitary hormones.

METHODS AND RESULTS.

(a) Bromide Distribution in Tissue.

(1) Radio-active bromine method:

An activity of 10 μCi of Br^{82} was injected into the peritoneal cavity of each of six guinea-pigs. After 24 hours the animals were anaesthetised and blood was taken by heart puncture. The animals were then dissected and the activity of the various organs and tissues was measured using a whole body monitor. The resulting grades of activity

are shown in Table 7.

(2) Chemical method:

15 adult guinea-pigs of both sexes were used. The animals were anaesthetised, blood was taken by heart puncture for further investigation, and they were then dissected. Various tissues and sera were analysed for bromide content. Rabbits and rats were treated in the same manner, and human organs were obtained following post mortem examination, and similarly studied.

The results of these analyses are shown in Table 8.

(b) The Pattern of Accumulation of Bromide.

Guinea-pigs were divided into eight groups with 2 to 8 animals in each group; varying amounts of bromide (K Br and Na Br) from 10 p.p.m. to 400 p.p.m. were added to the drinking water of each group. A control group was given distilled water, while food was common to all groups. The animals were kept in this condition for two months, after which they were anaesthetised, blood was taken by heart puncture, and they were dissected, and lung, kidney, heart, liver, brain, muscle and serum were analysed for bromide content (see Table 9).

(c) The Effect of Fluoride on Normal Bromide Level.

Three guinea-pigs were kept for 5 weeks and 5 p.p.m. of fluoride (Na F) was added to their drinking water. A control group was also run at the same time. The animals were then killed and dissected, and the bromide levels were measured in the lung, kidney, heart, liver, brain, muscle and serum. The

resulting figures are shown in Table 10.

(d) Bromide in the Human Pituitary and Brain.

(1) Using the chemical method, the bromide level in several pooled human pituitaries was estimated. The glands were collected over a period of 7 days following post mortem examination. The resultant level of bromide found, as seen in Table 8, is not high compared with that of other tissues.

(2) Homogenate was obtained from about 150 human pituitaries and this was dialysed against distilled water, which was repeatedly tested for bromide until no bromide was detected. The homogenate itself was then tested for bromide, and the result was negative.

(3) A human brain was dissected into the major parts, and samples of tissue from these various regions were analysed for bromide. The result of these findings is shown in Table 11.

DISCUSSION.

(a) Distribution of Bromide in Tissues.

In considering the uptake of Br^{82} by various tissues in guinea pigs it must be stated at the outset that for technical reasons not all the tissues listed were examined in each guinea pig. It is therefore possible that, had more specimens been measured, the average figures in some instances might have been different. Secondly, there was a considerable variation in the uptake of Br^{82} by the same tissue in different animals (see Table 7).

TABLE 7

Br^{82} UPTAKE BY VARIOUS TISSUES IN GUINEA-PIGS

Tissue	μ Ci/1 g wet tissue in individual guinea-pigs						Average m-μ Ci/g tissue
	1	2	3	4	5	6	
Pancreas	-	.0246	-	.0121	-	-	18.35
Thyroid	.0166	.0250	.0200	.0139	.0115	-	17.40
Lung	.0190	.0154	.0147	.0152	.0159	.0159	16.01
Kidney	.0162	.0132	.0122	.0117	.0141	.0072	12.43
Heart	.0100	.0109	.0091	.0109	.0089	.0096	9.90
Gonads	-	.0088	.0087	.0097	.0114	.0106	9.84
Spleen	.0099	.0104	.0081	.0093	.0104	-	9.62
Liver	-	.0092	.0080	.0078	.0085	.0084	8.38
Diaphragm	.0016	-	.0019	.0082	.0016		8.32
Adrenals	.0069	.0076	.0092	.0073	.0071		7.62
Bone	.0071	-	-	.0057	-	-	6.40
Skin	.0059	-	-	.0045			5.20
Brain	-	.0052	.0050	.0034	.0054		4.75
Muscle	.0041	.0042	-	.0037	.0046	.0043	4.18
Fat	-	-	.0024	.0024			2.40
Serum (1 ml)	.0260					.0259	25.95
Whole blood (1 ml)	.0230					.0220	22.50

TABLE 8

BROMIDE IN TISSUES

(µg/1 g wet weight)

Tissue	Guinea-pig	Man	Rabbit	Rat
Stomach	12.6 - 17.2 *(4)	-	-	-
Lung	4.0 - 10.4 (12)	4.7 - 12.0 (7)	3.2 - 7.0 (2)	5.2 - 8.8 (9)
Kidney	4.5 - 7.0 (11)	4.1 - 8.2 (7)	3.2 - 8.0 (4)	4.3 - 4.5 (2)
Heart	4.4 - 7.0 (9)	3.0 - 9.0 (7)	3.5 - 4.0 (3)	3.0 - 4.0 (8)
Liver	3.2 - 6.6 (13)	1.8 - 5.5 (12)	1.0 - 3.6 (7)	1.8 - 2.9 (8)
Spleen	3.4 - 6.1 (8)	5.0 - 10.0 (8)	-	3.2 (7)**
Brain	2.5 - 5.8 (12)	1.6 (1)	3.0 (2)	2.2 - 2.5 (2)
Muscle	2.6 - 5.2 (11)	-	1.0 (1)	1.2 - 2.2 (9)
Adrenals	3.3 (6)			
Thyroid	-	4.5 - 5.2 (3)	-	-
Pituitary		5.2 (7)**		
Gonads	-	-	-	3.5 (1)
Prostate		3.2 (1)		
Serum µg Br/ml	4.5 - 11.0 (18)	0 - 20.5 (96)		

* Figures in brackets represent number of specimens.

** Pooled specimens.

TABLE 9

CUMULATIVE EFFECT OF BROMIDE FEEDING

(2-8 guinea-pigs in each group)

ppm Br in drinking water	Thyroid	Lung	Kidney	Heart	Liver	Brain	Muscle	Serum
		(µg Br/1 g w.w.)						(µg Br/ml)
Normals		*7.0	5.9	5.9	5.5	4.0	3.5	7.7
10		21	19	16	16	16	13	27
15		28	29	23	14	-	23	35
20		38	32	32	29	28	36	42
30		40	42	38	49	-	38	73
80		50	54	49	49	43	42	-
200		107	137	131	86	72	66	206
300		145	146	147	150	112	88	305
400	200	169	160	171	164	140	102	380

* All figures are calculated averages.

TABLE 10

THE INFLUENCE OF FLUORIDE FEEDING ON BROMIDE DISTRIBUTION IN GUINEA PIGS

	Normals		5 ppm of Na F in drinking water for about 5 weeks	
Organ	No. of specimens	Range (µg Br/1 g w.w.)	No. of specimens	Range (µg Br/1 g w.w.)
Lung	12	3.7 - 10.4	3	2.2 - 2.6
Kidney	11	4.5 - 7.0	3	1.8 - 2.1
Heart	9	4.4 - 7.0	2	2.3 - 2.5
Liver	12	3.2 - 6.6	3	1.9 - 2.2
Brain	12	2.5 - 5.8	3	1.0 - 1.3
Muscle	11	2.6 - 5.2	3	1.0 - 1.2
		(µg Br/ml)		(µg Br/ml)
Serum	18	4.5 - 11.0		5.0 - 6.0

TABLE 11

BROMIDE IN HUMAN BRAIN

No.	Region of Brain Examined	µg Br/g wet wt.
1.	Cranial Nerves	1.8
	Cerebellum:	
2.	Tonsil, Flocculus, Inf. Vermis, etc.	1.9
3.	Peduncles	0.9
4.	Cortex	1.9
5.	Medulla	1.3
	Midbrain:	
6.	Brachia, Tectum, Pineal Colliculi and Deeper Tracts	1.5
7.	Substantia Nigra and Red Nucleus	1.8
	Pons:	
8.	Anterior	1.3
9.	Post. - Tracts	1.3
10.	Medulla Oblongata	0.9
	Cerebrum:	
11.	Visual Cortex	1.8
12.	Auditory Cortex	1.9
13.	Frontal Cortex	1.5
14.	Motor and Pre-Motor Area	1.9
15.	Sensory and Pre-Sensory Area	1.8
16.	Fimbria, Dentate Gyrus, Uncus Hippocampal Gyrus	1.3
17.	Insula	2.4
18.	Other Cortex (Whole Areas)	2.0
19.	Corpus Callosum, Fornix and Cingulum	1.8
20.	Forceps, Tapetum and Corona Radiatum	1.3
21.	Basal Nuclei	1.9
22.	Association Fibres, Commissural, Projection	1.1

Bearing in mind the above, it was found that 3 or 4 tissues had a relatively high Br^{82} uptake, viz. pancreas, thyroid, lung and kidney, whereas low uptakes were seen in skin, brain, muscle and fat. The remaining tissues examined all showed an uptake similar to each other between these two extremes. One point of interest is that the bromine uptake of bone was higher than that of either brain or muscle tissue.

Although the highest average uptake was in the pancreas, only two specimens of this organ were measured; one was relatively high, and the other much lower. However, in 2 cases where Br^{82} uptake was measured in both pancreas and thyroid, it was found that Br^{82} uptake by the thyroid was consistently higher than by the pancreas (see Table 7). Generally, the uptake by the thyroids was variable in individual guinea pigs and ranged from 25.0 to 11.5 m-μCi/g tissue. On the other hand, the uptake of the lungs was almost uniform in all animals. The variation in the thyroid uptake could have been due to the individual variation of the guinea pigs or to the relative iodide level in the body (see Chapter on the Thyroid). Further, an examination of the results of this experiment indicates that the uptake by the liver is approximately half that of the lung.

Finally, it could be stated that, although there is a definite difference in the degree to which various organs take up bromide (Br^{82}), even those tissues with the highest uptakes do not exceed that of blood, i.e. the tissue/plasma ratio does not exceed unity, although it is possible that the thyroids in some animals might have achieved unity. Unfortunately, it was not possible to measure the blood and serum from

all animals.

In comparing the above results with those of other authors (see Table 12) it would appear that, although different workers found significant variations in the uptake of Br^{82}, there are certain common features to all. For example, all workers found in all species the lowest Br^{82} uptake to be in brain and muscle, while, with the exception of Cole and Patrick (28), the thyroid, stomach and spleen were the tissues with the highest uptake. That of the adrenals was very similar in most cases, and while Perlman et al. (54) and Baumann et al. (55) found that the Br^{82} uptake of the thyroid was greater than that of serum, Mack and Shipley (27) found the reverse to be the case.

Despite certain variations (e.g. Cole and Patrick's pancreatic findings) a similar general pattern of Br^{82} uptake in the tissues was found by the previous workers and in the present investigation.

The bromide levels in the various tissues of normal guinea pigs which were estimated by chemical means show variations in individual animals (see Table 8). Although fewer tissues were examined compared with the Br^{82} uptake method, a similar pattern can be seen, viz. lung, kidney and heart with a relatively higher bromide content than liver, brain and muscle. However, in this experiment the stomach was also examined and was found to have by far the highest bromide content, and was in fact the only tissue in which the tissue/serum ratio exceeded unity. This supports the findings of Mack and Shipley (27), Gamble et al. (57) and others that the stomach concentrates more bromide than any other tissue.

TABLE 12

Br^{82} UPTAKE IN VARYING AMOUNTS CARRIED OUT BY DIFFERENT AUTHORS

Author	Species	Tissue
Perlman et al. (1941)	Rats	Thyroid > whole blood > liver = kidney = salivary gland = pituitary > adrenals > brain.
Mack and Shepley (1952)	Rats and rabbits	Serum > stomach > thyroid > gonads > kidney > spleen > pituitary > small intestine > adrenals > liver > large intestine > brain > muscle.
Baumann et al. (1956)	Rats	Thyroid > blood > lung > muscle
Cole and Patrick (1958)	Rats	Intestine > kidney > spleen > gonads > thyroid = adrenals > pancreas > heart > liver > brain.
Present investigation	Guinea pigs	Serum > pancreas > thyroid > lung > kidney > heart > gonads > spleen > liver > diaphragm > adrenals > bone > skin > brain > muscle > fat.

In comparing the results of different authors of the bromide content in various tissues of several species, two main factors must be kept in mind: firstly, the different chemical methods of bromide estimation in biological material, and secondly, the variation due to different dietary habits. Table 13 shows the values obtained by different workers, and the following observations could be made: the results obtained by Damiens are very similar to those of the present investigation except in the human, where the findings were somewhat lower. On the other hand, by comparison the results of Bernhardt and Ucko are high, particularly for the pituitary; this could have been due to the analytical method used or to estimating the bromide content of tissue from animals with some degree of bromide intoxication, or a combination of both factors. However, the difference in the findings between the liver and spleen on one hand and the pituitary on the other is very hard to explain if the same animals were used for both, but this is not known.

Although Picussen and Roman (53) obtained 25 µg Br/g wet weight in whole white mice, this figure may not be unusually high, as the bromide estimation in whole animals includes organs with a very high bromide content such as the stomach and its contents, while the diet may also have been a contributory factor. The results obtained by Ucko (16) in the tissues of the cow are within the range obtained in the present investigation, in which Hunter's method has been used.

The cumulative effect of bromide intake shows that the level of bromide in the tissues and serum is dependent on the bromide content of the diet. Taking into account the individual variation of the

TABLE 13

µg OF BROMIDE/g WET TISSUE. DERIVED FROM THE LITERATURE

Author	Species	Tissues									
		Adrenals	Heart	Kidney	Stomach	Lung	Liver	Spleen	Thyroid	Gonads	Pituitary
Damiens (1920)	Chicken, Pheasant, Pigeon	(Range from 0.5 to 8.4)									
	Cattle	1.3				4.2					
	Human			1.4-2.5		(1.4 - 2.5)					
Bernhardt and Ueko (1926)	Dog	50									125
	Human	14-18					(6.0 - 14.0)				150-200
Picussen and Roman (1929)	Mice	Content of whole white mice 25 µg/g w.w.									
Ueko (1936)	Cow	6.2							7.5	6.1	6.1
Present Investigation	Guinea-pigs	3.3	4.4-7.0	4.5-7.0	12.6-17.1	4.0-10.4	3.2-6.6	3.4-6.1	-	-	-
	Human	-	3.0-9.0	4.1-8.2	-	4.7-12.0	1.8-5.5	5.0-10.0	4.5-5.2	-	5.2
	Rabbit	-	3.5-4.0	3.2-8.0	-	3.2-7.0	1.0-3.6	-	-	-	-
	Rat	-	3.0-4.0	4.3-4.5	-	5.2-8.8	1.8-2.9	3.2	-	3.5	-

animals, examination of the results shown in Table 9 reveals a certain pattern of this cumulative effect. Firstly, in almost all tissues the increase in the bromide level was roughly proportional to the dose given, although in the lung, kidney and heart the large doses (viz. 300 to 400 p.p.m.) did not give rise to a corresponding increase of the bromide level in these tissues. Secondly, at the high bromide doses the differences between the bromide levels in the tissues tended to decrease, with the exceptions of muscle and, to a lesser extent, brain. Thirdly, at the high doses where the thyroid content was also measured this tissue proved to have a far higher bromide content than any other tissue examined. Finally, none of the tissues examined showed a greater bromide content than serum, and the tissue/serum ratio did not exceed unity. Generally, this ratio decreased with increased dosage of bromide in all tissues (see Table 14), and although at 400 p.p.m. the ratio in the thyroid was highest compared with other tissues, it was nevertheless well below unity.

It can be concluded from this experiment that the prolonged administration of high doses of bromide alters the tissue/serum ratio by lowering it.

The figures in Table 10 show that fluoride affected the distribution of bromide in the tissues of guinea pigs. In the tissues examined the level of bromide in tissues of animals which were on fluoride was significantly lower compared with untreated animals. To prevent any inter-batch difference during the chemical estimation, though this has been found to be small when it does occur, controls and

TABLE 14

THE EFFECT OF INCREASING DOSES OF BROMIDE ON THE TISSUE/SERUM BROMIDE RATIO

ppm Br in drinking water	Thyroid	Lung	Kidney	Heart	Liver	Brain	Muscle
Normal		0.90	0.77	0.77	0.71	0.52	0.45
10		0.78	0.70	0.59	0.59	0.59	0.48
15		0.80	0.83	0.66	0.40	-	0.66
20		0.90	0.76	0.76	0.69	0.67	0.86
30		0.55	0.58	0.52	0.67	-	0.52
80		-	-	-	-	-	-
200		0.52	0.67	0.64	0.42	0.35	0.32
300		0.48	0.48	0.48	0.49	0.37	0.29
400	0.53	0.44	0.42	0.45	0.43	0.37	0.27

fluoride animals were tested in the same batch on three occasions. The significance of the differences obtained between control and fluoride animals may not be as great as it appears, since only 3 animals on fluoride were tested. Nevertheless, the findings merit attention.

Examination of the bromide level in 7 human pituitaries showed that the bromide content was not higher than in other tissue (see Table 8). Extensive dialysis of homogenate of human pituitaries and the subsequent testing of the homogenate for bromide indicated that bromine is unlikely to be bound to a protein or any undialysable organic molecule. It does not, however, exclude the possibility of a small dialysable organic molecule. Nevertheless, the relatively low bromide content in the pituitary would tend to reject this possibility because it would be expected that if a particular organ concentrates bromide, and therefore has a tissue/serum ratio well above unity, this would probably require some bromide binding within the cells. Since the bromide level in the pituitary was low, such organically bound bromine is therefore unlikely. In addition, the bromide estimation of various parts of a human brain showed (see Table 11) not only that the bromide level in the brain is very low, but also that there is no significant difference in the bromide level between the various parts of this organ.

In conclusion, it must be stated that the present investigation gave no evidence to support the claim of Zondek and Bier (45, 46) concerning the possible physiological function of bromide in the

pituitary and brain. The tissue/serum ratio of bromide in the human pituitary was calculated and found to be 0.50, a somewhat higher figure than Mack and Shipley (27) reported for the rabbit and rat.

CHAPTER III

THE THYROID AND BROMIDE

The thyroid gland has the capacity for concentrating iodine and maintaining a concentration gradient of 25 - 500 times that of the serum under various conditions (83, 84). The fact that almost all of the iodine in the normal gland is organically bound (85) might suggest that this concentrating capacity is mainly dependent upon the gland's ability to convert inorganic iodide to diiodotyrosine and thyroxin. Although this view is supported by the rapid rate at which an injected dose of I^{131} is organically bound by the gland (85), there is a considerable amount of evidence otherwise (86, 87).

Franklin et al. (88) showed that the capacity of slices of sheep thyroid to form diiodotyrosine and thyroxin was inhibited in the presence of thiourea and thiouracil (10^{-3}M), but these drugs did not prevent the collection of inorganic iodide in the tissue. This has been confirmed by many other workers (83, 84, 89, 90, 91).

The antithyroid drugs of the thiourea group have provided a means of "dissecting" for study the systems of the thyroid involved in iodide trapping on the one hand, and hormone synthesis and release on the other (91). It is mainly this iodide trapping mechanism which has been the subject of many studies, namely the interference of other ions in this system. It has been reported that trapping rather than binding is the rate limiting step of thyroidal iodine accumulation (92).

Bromine has often been thought to play an important part in the physiology of the thyroid gland. Bernhardt and Ucko in 1926 (14) reported that the thyroid contains more bromine than any other tissue. This has subsequently been supported by other workers (93, 94).

Perlman et al. in 1941 (54) using radio-active bromine (Br^{82}) found the highest uptake by the thyroid glands in rats and guinea-pigs. Hypertrophied glands produced by treatment with thyrotropic hormone showed about 30% increase in the Br^{82} uptake. Similar results were obtained by Baumann et al. in 1956 (55) who showed that from 10 - 80% more Br^{82} was present per gram of thyroid than per gram of blood.

Baumann et al. (95), working with rabbits, produced experimental evidence that hyperplastic thyroid tissue of low iodine and colloid content has more bromine than the circulating blood, while in fully iodized thyroids rich in colloid no more bromine is found than is present in the blood. They suggested that the bromine content of the thyroid may be explained on the assumption that thyroid tissue can distinguish only imperfectly between iodine and bromine. Glands which are hyperplastic due to a deficiency of iodine will, in the absence of iodine, seize bromine instead. (There is 10 - 100 times as much bromine as iodine in food and water.) When iodine is supplied to animals with hyperplastic thyroids they quickly lose the accumulated bromine.

This assumption that bromine can be selectively absorbed by the thyroid, but cannot substitute iodine in the hormone synthesis, is supported by work of Peterson and Yomamoto (96) who reported that a

hyperthyroid patient's thyroid concentrated 17 - 20% of the Br^{82} tracer dose, and 55 - 70% of I^{131} tracer dose. At 24 hours they found 57% of the serum I^{131} was organically bound, while less than 0.4% of the serum Br^{82} was organically bound. These data strongly suggest that the thyroid significantly concentrates but does not organically bind the bromine.

It seems that bromine will concentrate in an iodine deficient thyroid, also that if bromine is administered continuously in large doses, it will decrease thyroidal iodine. It has been found (97, 98) that continual feeding of sodium bromide to young rats will decrease thyroidal iodine with resultant goitre. There is evidence, however, indicating that this action is common to all halogens.

It has been shown (99) that sodium fluoride administered to rats for several months caused the development of hyperplastic thyroids as much as eight times the normal size. Fluoride has also been reported to cause goitre in pigs (100). Thyroid hyperplasia has been caused in rats on a diet containing 1 - 4% sodium chloride (101). The thyroid iodine was reduced to one third of the amount in the control animals.

Moreover, Williams et al. (102) found that sodium chloride, sodium bromide and sodium fluoride caused an augmentation of the goitrogenesis of thiouracil and propylthiouracil in rats. Goitres were also produced in animals receiving one of the halides and one of the thiouracils, when the concentration of the latter alone was too small to produce goitres. Fluoride was found to be several times more active

than either of the other compounds, while bromide was more effective than chloride. They suggested that these variations in results may be attributed to differences in permeability of cell membranes to these ions.

On the other hand, the administration of large quantities of potassium iodide to rats given thiourea has not produced a significant effect on goitrogenesis (103), but when the intake of iodine has been low, the amount of thiouracil necessary to produce a goitre has been shown to be decreased. Further, it has been demonstrated in rats that goitrogenesis was greater with sodium bromide given with propylthiouracil than with either one alone or both combined with potassium iodide.

It appears, therefore, that the thyroid gland selectively filters all halogens, especially if iodine deficiency is present; however, iodide is the only halide that is used in thyroid hormone synthesis. Non-iodide halides administered in large doses have a mass-action-like effect, both decreasing trapping and increasing discharge of the iodine normally ingested and this eventually leads to hyperplasia and goitre. In addition, the action of these non-iodide halides may also lead to competition of these ions for reabsorption by the renal tubules thereby producing an increased rate of excretion of iodide (102).

Fluoride probably produces its effect by mechanisms additional to the foregoing ones, since it inhibits many enzymatic reactions throughout the body.

This competitive action of non-iodide halides is supported by reports that these ions may interfere with radio-active iodine uptake by the thyroid. Thus, it was shown by Petersen and Yamamoto (96) on 28 euthyroid patients that bromide depressed thyroidal I^{131} uptake. This was supported by Glode et al. (104) who showed on rats which were injected with 15 mg of bromide peritoneally daily for 3 - 270 days, that the longer bromide was administered, the lower was the I^{131} uptake. In contrast, the I^{131} uptake measured on rats which had been on water containing bromide (0.15 mg/cc) was higher than in the control animals used; however, this was found to be due to increased thyroidal mass, not due to the increase of any specific activity (104). The glands showed goitrous conditions, which is another indication that bromide or any other non-iodide halide will interfere with iodine metabolism if administered over a long period and in relatively large doses.

Although there is sufficient evidence indicating that non-iodide halides interfere with iodine metabolism, there have also been a few reports which denied this. It has been shown (84) that bromide doses over 500 times that of injected iodide will not cause significant iodide displacement. This has been supported by another experimental study. Wyngaarden et al. (105) failed to show that the administration of fluoride, chloride or bromide in doses of 0.1 M had any effect upon I^{131} accumulated within propylthiouracil treated thyroids. In all these cases, however, only a single dose of these ions was used, and therefore such results could be expected.

Several workers (27, 28) failed to demonstrate preferential uptake of Br^{82} by the thyroid of rabbits and rats. It has been suggested by Mack and Shipley (27) that the lack of such selective concentration of Br^{82} in their experimental animals might depend on either an accidentally low level of thyroid activity in the animals, or the existence of a state of relative iodine saturation. The latter suggestion seems the more likely, as the evidence of Baumann et al. (95) shows that a significant substitution of bromine for iodine requires the presence of a pre-existing iodine depletion.

Thus it could be stated that in considering the effect of non-iodide halides on iodine metabolism the duration of administration of these ions is most important.

Finally it should be mentioned that there is evidence strongly suggesting that not only all halogens, but all elements of the 7th periodic group are selectively filtered by the thyroid.

It has been demonstrated (106) that the thyroid glands of guinea pigs and dogs which have been injected with manganese chloride will store 50 and 49 times respectively as much manganese as the control animals. It was found that the thyroidal concentration of radioactive manganese (Mn^{54}) was about 10 times that of serum two hours after injection (55).

Rhenium has been shown to be concentrated by the thyroid of rats from 25 - 100 times more than by any other tissue (107) after injection of radioactive rhenium (Re^{186}). Also astatine (108) and technetium (109) have been found to be preferentially concentrated by the thyroid.

The experimental evidence indicates that the ability of the thyroid to filter and concentrate elements of the 7th periodic group is greater for those of high atomic weight than for those of low atomic weight (55).

The questions now arise, how does the thyroid differentiate between the above elements and all others, and what is the mechanism of action of these interfering elements?

It must be stated from the outset that little is known which can answer these questions, though there is some suggestive evidence which may eventually lead to the understanding of these problems.

Baumann and Metzger (110) suggested that the behaviour of these elements is due to the similarity in the arrangement of their electrons. Astatine, bromine, chlorine, fluorine and iodine have 7 electrons in the outer shells, consisting of 2 in the s and 5 in the p orbitals. On the other hand, manganese, technetium and rhenium also have 7 electrons in their outer shells, but Mn and Re have 5 electrons in the d and 2 in the s orbitals, and Tc has 6 electrons in the d and 1 in the s orbitals. Wyngaarden et al. (105) failed to flush out accumulated I^{131} in "blocked" thyroid by doses (0.1M) of fluoride, chloride and bromide, but he showed that halogen oxides at the same concentration are more effective: perchlorate, periodate, iodate and chlorate caused a quantitative discharge of I^{131} in the initial 15 minute period. Biiodate and hypochlorite caused an incomplete discharge, and bromate was negative.

Perchlorate was shown to be most potent in preventing the collection of iodide by the thyroid and thus causing goitre (91, 105, 111).

Perchlorate reduces simultaneously the concentration gradient and inhibits iodide uptake (88, 106). Although the mechanism of its action has not been established, there is some evidence suggesting that a competition for certain sites in the thyroid cell is involved (105). This assumption is supported by the fact that perchlorate concentrates in the gland (112) and by the fact that the interference with iodide collection caused by perchlorate can be overcome by the administration of excess iodide (105).

The competition between perchlorate and iodide may be due to their similar monovalency and size. This is supported by the findings that monofluorosulphate and fluoroborate ions which are very similar in size and are iso-electronic with perchlorate, decrease the iodide accumulation in the thyroid to the same extent (112).

It has been suggested by Anbar and Guttmann (112) that the trapping mechanism in the thyroid involves a factor which scarcely distinguishes between different anions having the same charge, and comparable volume. Such a factor is probably a protein with a special arrangement which traps only those ions which fulfil these requirements. The mode of action of these ions upon the mechanism of iodide collection by the thyroid must await the full understanding of the mechanism by which the thyroid is able to accumulate inorganic iodide, and maintain a concentration gradient over the serum (105). It must, no doubt, involve an active transport of iodide across the cell membrane (113) and once in the cell is probably temporarily bound to some organic molecules, most likely to a protein. There is evidence which indicates that the

presence of intact cells is necessary for iodide concentration, as it has been shown that homogenization destroys this activity, most likely due to the destruction of cell membranes (114, 115, 116). An interesting exception to the requirements for cellular integrity has been found in certain fish (salmon, rainbow trout, etc.) with a serum protein which binds iodine relatively strongly (117).

In conclusion it could be stated that the obvious problem is to explain the different actions of the elements of the 7th periodic group and the halogen oxides. In the absence of any definite experimental evidence one can only speculate as to the answer. It is possible that there are two different levels or sites where the above ions could interfere by competing with iodide. Very recent work (118) suggests that there are two pools of iodide in the thyroid; the first consists of "trapped" iodide, and this is used very quickly for thyroid hormone synthesis, while the second iodide pool may be derived from deiodination of some of the monoiodotyrosine (M.I.T.) and deiodotyrosine (D.I.T.) which are thought to be the first products of thyroid hormone synthesis (119).

It has been shown (119) that some of the iodide in the thyroid cannot be discharged with perchlorate and it seems highly likely from experimental evidence that this iodide is from the "second pool".

It is possible to speculate from this that the relatively fast-acting halogen oxides interfere by competing for some sites during the "trapping" of iodide, i.e. in the first iodide pool (although the negative bromate remains unexplained) and the somewhat slow-acting ions such as bromide interfere with the iodide of the second pool, possibly by a simple mass action.

PRESENT INVESTIGATION

AIM.

Evidence has been presented that if bromide or any other halide is administered continuously over a relatively long period, it will interfere with iodine metabolism. The clinical importance of this interference is its possible adverse effect on thyroid function tests.

An experiment was set up to determine whether bromide or fluoride administered over a relatively short period (approximately five weeks) would have any effect on thyroid function; if so, it would be an adverse factor in the estimation of protein bound iodine in patients who had been on some bromide medication, including "Relaxa-Tabs", or who had consumed relatively small amounts of fluoride.

METHODS AND RESULTS.

The guinea-pigs were divided into three groups of 3 to 4 animals:

Group A - Control - Normal food + distilled water.

Group B - Bromide - Normal food + 400 p.p.m. of bromide (NaBr) in drinking water.

Group C - Fluoride - Normal food + 2 p.p.m. of fluoride (NaF) in drinking water.

(A low concentration of fluoride was used because of its possible effect on the enzyme system in the body.)

Any effect of bromide or fluoride on thyroid function may manifest itself by interference with (a) the iodide concentrating mechanism, (b) thyroid hormone synthesis and (c) the binding capacity of the serum proteins. Therefore after five weeks three standard tests were performed: (i) I^{131} uptake, (ii) protein bound iodine (P.B.I.^{131}) and (iii) T_3 resin uptake.

1. Radio-active iodine uptake.

The animals of all three groups were treated in the same way. An activity of 5 µCi of I^{131} was injected into the peritoneal cavity of each guinea-pig. At three hour and 24 hour intervals following injection the radioactive measurements were made in vivo by placing 3 of the animals from each group in a specially designed plaster-cast restrainer with a lead shield leaving only the thyroid zone exposed. A scintillation detector with a sodium iodide crystal was used. This was placed at a distance of 30 cm from the thyroidal area in each case. A standard of 5 µCi of I^{131} in a bottle was used as the 100% reference. The fourth guinea-pig was measured for radioactivity in the same way, except that the timing was in half-hourly intervals up to three hours.

Blood was then taken from all animals by heart puncture for further examination, mainly P.B.I.^{131} and T_3 uptake tests. The radioactivity due to protein bound radioiodine is insufficient to affect the result of the T_3-resin uptake. The guinea-pigs were killed under anaesthesia and the thyroids then dissected and fixed in formalin for histological examination.

2. Radio-active protein-bound iodine.

For this experiment the method of Silver (120) was used. In principle it involved the following: blood was collected 72 hours after the intraperitoneal administration of 5 μCi of radio-iodine. The serum proteins were precipitated with 10% trichloracetic acid, washed with distilled water and then dissolved in 2.5 N NaOH. The radio-activity was expressed as a percentage of the administered dose per litre of serum and indicates the amount of I^{131} labelled thyroxin, i.e. the rate of production of hormone by the thyroid gland.

3. Resin uptake of I^{131}-Tri-iodothyronine (T_3 uptake).

The method of Nava and De Groot (121) was used, the principle of which is: I^{131} labelled tri-iodothyronine is added to serum and resin and these compete for the tracer. The amount taken up by the resin is inversely proportional to the binding capacity of the serum proteins for the thyroid hormones.

The results of the above experiments are shown in Table 15, Figure 4.

DISCUSSION.

It is difficult to assess whether there was any significant decrease in the three hour I^{131} uptake in the bromide group considering that only three animals were measured, but it appears that there may have been some lowering effect. As shown in Figure 4 there was no

significant difference during the first three hours in the I^{131} uptake rate between the control and the bromide treated guinea-pig.

The P.B.I.^{131} results show, on the average, an increase in the bromide group as compared with the control. Since there was no difference between these two groups in I^{131} uptake, the possible increase of P.B.I.^{131} in the bromide group might suggest the stimulating effect of bromide on the enzyme systems of the thyroid gland. In the fluoride group, however, the result was suggestive of the lowering effect of fluoride on the production of thyroid hormone, and because the I^{131} uptake was normal in this group it is likely that any fluoride effect on thyroid function is exerted on the synthesis of the hormone itself in the gland.

Considering that only a relatively low concentration of fluoride was used, compared with bromide, the above results would support the previously mentioned suggestion that fluoride, in addition to its competitive action with iodide, will also affect enzyme systems in the thyroid gland.

The fact that only 3 to 4 animals were used in each group must be taken into consideration before any definite conclusions are drawn concerning the effect of bromide or fluoride on thyroid function.

That the serum binding capacity was not affected by the above ions was indicated by the T_3-resin uptake test, which showed no significant difference between the three groups of guinea pigs.

The histological examination of the thyroid glands of the animals showed no evidence of goitre.

TABLE 15

THE EFFECT OF BROMIDE AND FLUORIDE INTAKE ON THYROID FUNCTION IN GUINEA-PIGS

	No. animal	Control	Average	Bromide	Average	Fluoride	Average
3 hour thyroidal uptake of I^{131} % of A.D. of I^{131}	1	25.4		23.5		20.6	
	2	27.5	29.7	26.4	25.5	24.3	27.0
	3	36.3		26.7		36.1	
24 hour thyroidal uptake of I^{131} % of A.D. of I^{131}	1	25.3		28.7		20.4	
	2	28.7	22.6	24.6	27.1	26.0	22.0
	3	14.1		28.1		19.8	
PBI^{131}% of A.D./litre serum		2.3 3.5 7.0 13.2	6.5	3.0 8.0 13.2 15.7	10.0	1.8 3.5 6.0	3.8
T_3-resin uptake %		67.5 70.0 72.0	69.6	72.5 74.0 75.0 76.0	74.3	70.0 71.0	70.5

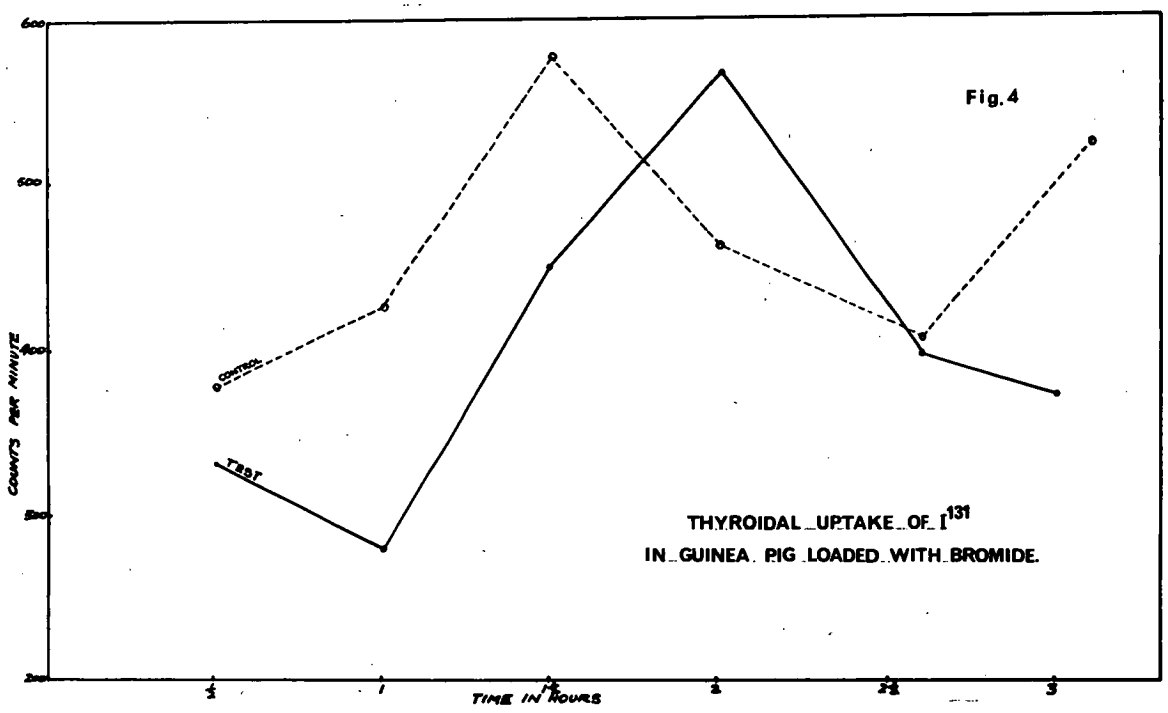


Fig. 4
COUNTS PER MINUTE
TIME IN HOURS
CONTROL
TEST
THYROIDAL UPTAKE OF I131
IN GUINEA PIG LOADED WITH BROMIDE.

CHAPTER IV

THE EXCRETION OF BROMIDE

The rate of bromide elimination from the body is very slow. Ucko in 1936 (16) reported that an increased amount of bromide in the blood and tissues can be found many months after the intake of bromide has ceased. Andrews (122) has shown that in humans it takes on an average between five and eight weeks to drop from a figure between 150 and 200 mg of bromide per 100 ml of serum back to normal (0.21 to 0.29 mg Na Br/100 ml).

Bromide, as chloride, is excreted mainly by the kidneys. Damiens in 1920 (52) reported the presence of bromide in human urine and claimed that normally from 0.3 - 0.75 mg of bromide/100 ml is excreted therein. Higher figures were obtained by Ucko in 1936 (16) who claimed that the bromide level in 24 hour urine specimens varied from 1.0 - 2.5 mg/100 ml. Söremark (123) found that nearly all radio-bromide administered was excreted by the urine while the excretion of bromide by the faeces was extremely low. The loss of bromide in the urine is found to be variable and not to correspond always with the serum bromide level so that while in general a high bromide level in urine is found when the level in serum is high, this is not invariably so (122).

It has been demonstrated (17) that, although bromide replaces chloride in the body, the total content of halides in the body remains relatively constant. If bromide is administered, a corresponding quantity of halide is excreted with the result that the total halide content is undisturbed. There have been, however, conflicting reports as to whether the kidneys distinguish between these two ions. The early workers, Frey (1932) (124) and Moller (1932) (125) believed that the kidneys do not distinguish between chloride and bromide. This has also been claimed in some textbooks (126), but is disputed by other workers, according to whom the kidneys seem to have a greater ability to excrete chloride than bromide (22, 23, 127).

Hastings et al. (22) showed that the excretion of chloride following a sodium chloride injection was practically equal to that following a similar injection of sodium bromide, and that the excretion of chloride and bromide was not proportional to the ratio of these ions in the serum.

Palmer and Clarke (23) presented data showing that the percentage of bromide in the urinary halides is lower than that in the blood halides. Further, that not only does the kidney excrete a smaller proportion of the bromide than of chloride, but the magnitude of this proportion appears to depend on the level of chloride intake. The ratio of the fraction of bromide to total halide in the urine to that in the blood is constant during a constant chloride intake. When the chloride intake is raised this ratio also rises, thus on a salt-poor diet it was 0.40 and on a high salt diet it was 0.70. They concluded

that this ratio depends upon the amount of halide being excreted and is independent of the blood bromide level, moreover, no relationship exists between urinary volume and bromide excretion. Following the chloride injection, the excretion of bromide and total halide, the urinary volume and the ratio of bromide to the total halide in the urine to that in the blood all rose; after an injection of urea, however, there was an increase in urinary volume comparable to that caused by the chloride injection, but the other figures showed no corresponding rise. Following the administration of chloride, the changes observed in the bromide excretion are therefore the result of a rise in halide excretion and not of changes in urinary volume (23). These results serve to explain the clinical observations that bromide intoxication responds to the administration of chlorides but not of other diuretics.

Mason (24) supported these findings when he reported his observation on dogs which had been given caffeine followed by 250 cc of 5% glucose intravenously, indicating that the resultant diuresis did not significantly alter the ratio of "replacement". He used the term "percentage replacement" (replacement of chloride by bromide) for halide distribution between serum and urine. If the kidney did not differentiate between bromide and chloride, as has been claimed by some, the replacements in the urine and serum would be equal in simultaneously collected samples, i.e. R urine/R serum would equal unity, where R is the ratio (expressed as a percentage) of bromide to bromide plus chloride, i.e. $\frac{Br \times 100}{(Br + Cl)}$. However he found that in dogs the ratio in replacement was 0.7. In the cases of patients, the ratios were more variable, and higher. The data indicated that for humans the value of the ratio of

replacement varies with the individual, but is nearly constant for a given individual. This individual variation might well have some bearing on the clinical observation that the amount of bromide used for producing bromism differs with the individual, as it is well known that the level of serum bromide at which symptoms of intoxication are manifested is variable.

Baumann et al. (95) subsequently showed that rabbits fed on a relatively low chloride diet had a high serum bromide level (2 -3 mg/100 ml), and on 50% dietary chloride increase, the blood bromide level dropped to about half of the original level. The variations of blood bromide on a constant diet are not more than 0.3 mg/100 ml.

The direct influence of chloride on the blood bromide level and its excretion was also reported by Evans (17) who suggested that, although after a single injection of bromide the kidneys will excrete a corresponding quantity of halide, the proportion of bromide in the excreted halide will be somewhat smaller than found in the serum; therefore it will take a relatively long time for all bromide to be excreted. However, if large doses of chloride are administered after the injection of bromide, the total output of halide will be increased considerably. Bromide will still be only a small proportion of the total halide excreted, but, as a result of the increased total output, the absolute quantities of bromide excreted will be increased, and bromide will be completely eliminated from the body more quickly.

Bodansky and Modell (128) have also demonstrated that there is a preferential excretion of chloride by the kidney at the expense of

bromide, and claimed that this is an important factor in the accumulation of bromide in the body. They suggested that the preferential excretion of chloride appears to depend upon the extent of reabsorption in the tubules. According to them, chloride and bromide are passed into the glomerular filtrate in an unselective manner, but bromide is reabsorbed much more quickly than chloride by the renal tubules. The result is that the proportion of bromide to total halides is less in the urine than in the blood. These workers supported their suggestion by the findings that the injection of mercurial diuretics caused a very large increase in the total halide excretion by the kidney, and also that the proportion of bromide to total halide in the urine so formed was increased and approached the proportion found in the blood. The current theory of the mode of action of mercurial diuretics is that they produce a diuresis by reducing the rate of tubular reabsorption of sodium with accompanying anions and water, without affecting the rate of glomerular filtration (129, 130).

The preferential excretion of chloride thus appears to depend upon the extent of reabsorption in the tubules. Theoretically, by producing a sufficiently high halide excretion a point could be reached where the ratio of bromide to total halide would be nearly the same in the urine as in the blood. Frey (131) has recorded values which indicate such a condition in the rabbit, but this point was not reached by Palmer and Clarke (23) who found that after the injection of sodium chloride into dogs the above ratio was about 0.70.

Excretion of Organic Bromine.

As discussed in Chapter V, most of the bromine ingested by

humans is in organic form, mainly as carbromal and bromvaletone. It is important therefore to have some knowledge of the way in which this organic bromine is excreted.

It is known that the hydrolysis of carbromal (bromo-diethyl-acetylurea) and bromvaletone (bromo-dimethylpropylurea) gives urea and a corresponding brominated fatty acid, i.e. bromo-diethylacetic acid or bromo-dimethylpropionic acid. The manner of degradation of the bromo-fatty acids with the release of inorganic bromide has not been established as yet (132). According to Stohr (133) most of the carbromal ingested by animals is excreted in three forms, viz. inorganic bromide, bromo-fatty acid and an ether soluble substance containing bromine, and very little is excreted unchanged via the kidneys.

Wollheim (134) compared the excretion of bromine in organic and inorganic combination after bromo-ureide intake. He found that only from 1.6 to 6.2% of the bromo-ureide bromine was excreted in organic combination (the figure depended upon which bromo-ureide was used). This was eliminated promptly within three days of ceasing bromo-ureide intake, whereas the rest of the bromide was excreted in the inorganic form with the usual slow elimination characteristic of this form of bromide.

Diurnal Rhythm.

It is known that varying quantities of urine and electrolytes are excreted by humans in a diurnal pattern (variation rhythm) (135, 136). In temperate climates about two-thirds of the excretion of water and

sodium occurs by day and there is a relative antidiuresis at night. This normal rhythm is well maintained even though the length of day is changed drastically or sleep is reversed (136).

According to MacFarlane (137) the diurnal rhythm is reversed in the dry tropics, and considerably less water and sodium are excreted by day than at night, especially during acclimatisation. The main excretion of sodium is nocturnal, providing that the night is cooler than the day.

There is some evidence showing that bromide is also excreted in a diurnal pattern. Ucko (16) reported that the bromide content in urine varied considerably in samples passed at different periods of the day. Söremark (123) using radioactive bromide in humans showed that bromide is excreted in a diurnal rhythm. He claimed that during sleep there was hardly any bromide excreted.

The Effect of Thyroid Hormone on Bromide Excretion.

There is mounting evidence that the thyroid hormone affects the renal function, i.e. the secretory function of the renal tubules is increased in hyperthyroidism and decreased in hypothyroidism (138, 139, 140). This has also been demonstrated in relationship to bromide excretion. Although some early workers (141, 142) claimed that when animals are fed on desiccated thyroid the blood bromide level rises, this has been disputed by Baumann et al. (95) who showed on the contrary that after feeding desiccated thyroid to animals the bromide level in blood decreased, but the fall did not occur until from two to three weeks after

medication and then the blood bromide slowly rose to normal. Also, when rabbits with hypertrophied thyroids were given iodide, excessive thyroid secretion occurred with a resultant gradual fall in the blood bromide level (from 35 - 50%) and again the decrease was not evident until about two weeks after the iodide was given.

The lowering of blood bromide either by feeding thyroid or by the excessive thyroid secretion of goitrous rabbits given iodide can be ascribed to the fact that the thyroid hormone acts on the kidney to increase renal function (143, 144), thus greater amounts of bromide as well as other salts are excreted.

Bromide Excretion Other Than Through the Kidney.

Although most of the excreted bromide is found in the urine, small quantities are excreted in the sweat, tears, saliva and gastric juice (17). Mason (24) found that human mixed saliva had a much greater replacement of chloride by bromide than the serum. He concluded therefore that one or more of the human salivary glands must preferentially secrete bromide to a marked degree. Söremark (123) claimed that the mean concentration of Br^{82} in human urine and saliva was about equal, and $1\frac{1}{2}$ times that of the blood level.

Nencki and Schoumow-Simanowski in 1894 (61) observed the presence of bromide in the gastric juice of dogs given bromide. Davenport and Fisher (1940) (58) have clearly shown that in the Pavlov pouches in dogs the rate of secretion of bromide is of the same order as the rate of secretion of chloride and the concentration of bromide in

the gastric juice is about 50% higher than in plasma. However, the volume of gastric juice in any short period could not be more than from 3 - 4% of the volume of the extracellular fluid. Hunter et al. (29) therefore suggested that, with only a 50% excess of bromide in gastric juice over the plasma level, this could not be regarded as likely to change the plasma level. Thus a fall in plasma bromide level of more than 1 - 2% following a very active period of gastric secretion would not be expected, and such an alteration is within the usual analytical variations. Therefore it could be stated that the gastric secretion is not a very significant factor in lowering serum bromide.

Bromide is also secreted in the milk and it easily crosses the placental barrier (17). Lynn et al. (145) reported that bromide is secreted in the milk of lactating dairy cows when the element is present in their diet, as a result of naturally occurring bromide in forage or grain, added sodium bromide, or inorganic bromides arising from methyl bromide fumigations. The amount of bromide in the milk is proportional to that in the diet and appears to be correlated to levels of bromide in the blood. A total diet containing 43 p.p.m. bromide resulted in 10 to 20 p.p.m. bromide in milk with no effect on production.

In conclusion, it could be stated that there are many ways by which bromide can be excreted from the bodies of humans and animals. However, the kidneys are by far the most important excretory organ, although the amounts of bromide eliminated from the body of lactating animals and humans is certainly significant.

PRESENT INVESTIGATION

AIMS.

(a) To investigate the bromide excretion rate of the "normal" human body. In this experiment, human subjects were chosen because of the clinical importance of this aspect and for the easier control of the collection of urine, etc.

(b) To study the effect of a dose of bromide on other serum and urinary electrolytes, mainly that of chloride, in view of the claim by Palmer and Clarke and others that chloride is preferentially excreted by the kidney.

(c) To determine whether bromide is excreted in the same diurnal pattern as chloride. It is known that chloride is excreted in a diurnal variation, and that bromide can replace chloride in the body, therefore the excretion pattern relationship between the two ions was considered to be of some interest.

METHODS AND RESULTS.

(a) Excretion Rate.

In these experiments, both radioisotopic and chemical techniques were used.

(1) Retention Test - using a whole body monitor:

The retention time of bromide in man was measured. For this

purpose 4 human subjects (3 males and 1 female) took an oral dose of radioactive bromine ($K\ Br^{82}$) of 1 μCi of activity, and the overall activity was measured at several intervals in the whole body monitor. The results of this experiment can be seen in Table 16 and Figure 5.

The following comments should be made with regard to these results:

(i) The reading immediately after the oral dose is on an average 8% lower than the values obtained between 1 and 3 hours after zero time. The measurement at one hour was therefore taken to represent the 100% retention value. The reason for the above is that there is greater accuracy in counting a dispersed source than a point source, and immediately after the oral intake of $K\ Br^{82}$ this is mainly confined to the stomach.

(ii) In the case of subject B, a one-hour measurement was not made, therefore the three-hour value was taken as 100%; this however did not give rise to any significant error, as the average retention at 24 hours of the four subjects was 98%.

(2) <u>The Bromide Excretion Rate of One Human Subject:</u>

The bromide level in the serum and urine of one human subject was estimated for several days before a single dose of potassium bromide 0.5 g was taken orally. Blood samples were then taken at two or more day intervals; pooled 24-hour urine specimens were collected daily for approximately five weeks, and the bromide level estimated in each.

The results, as indicated in Table 17 and Figure 6, showed the relatively slow excretion of bromide.

TABLE 16

RADIOACTIVE BROMINE RETENTION TEST IN THE HUMAN

(Using a whole body monitor following oral administration of -- 1 μCi of K Br^{82}

Subject	Sex	Age	Weight in kg	Time after dose	% retention	Biological half-life
A	F	19 yrs.	60.4	0 hrs. 1 hr. 3 hrs. 24 " 120 " 169 "	93.2 100 101.3 97.2 59.4 40.0	140 hrs.
B	M	38 yrs.	76.7	0 hrs. 3 hrs. 24 " 143 " 195 "	88.8 100 96.9 58.0 38.0	160 hrs.
C	M	25 yrs.	68.0	0 hrs. 1 hr. 24 hrs. 96 " 150 "	-- 100 100 63.0 40.0	124 hrs.
D	M	23 yrs.	79.0	0 hrs. 1.5 " 3.5 " 23.5 " 124 " 197 "	94.3 100 97.0 99.0 54.0 34.0	134 hrs.

Fig. 5

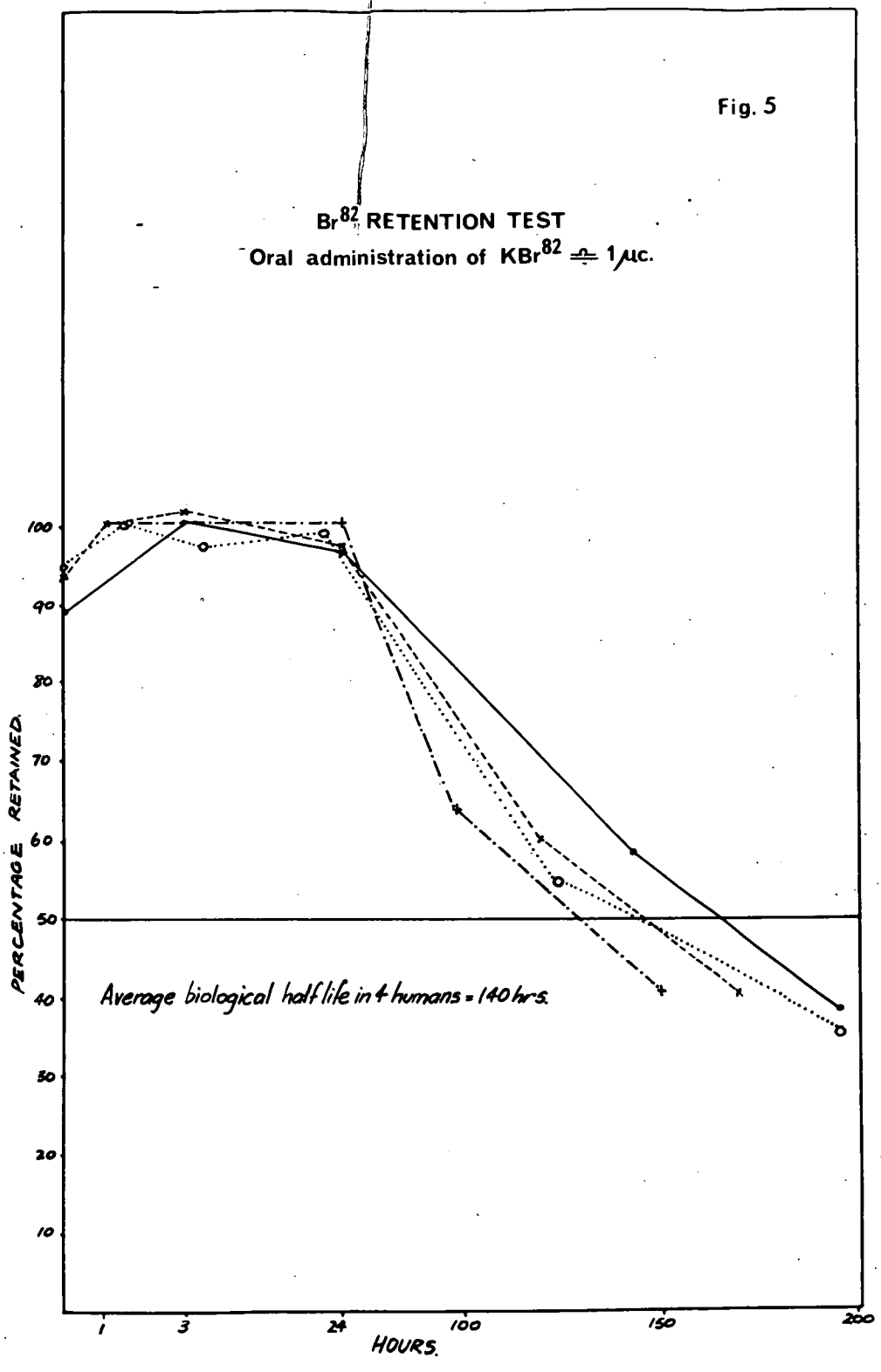

Br82 RETENTION TEST
Oral administration of KBr82 ≐ 1 μc.
PERCENTAGE RETAINED.
100
90
80
70
60
50
40
30
20
10
Average biological half life in 4 humans = 140 hrs.
1
3
24
100
150
200
HOURS.

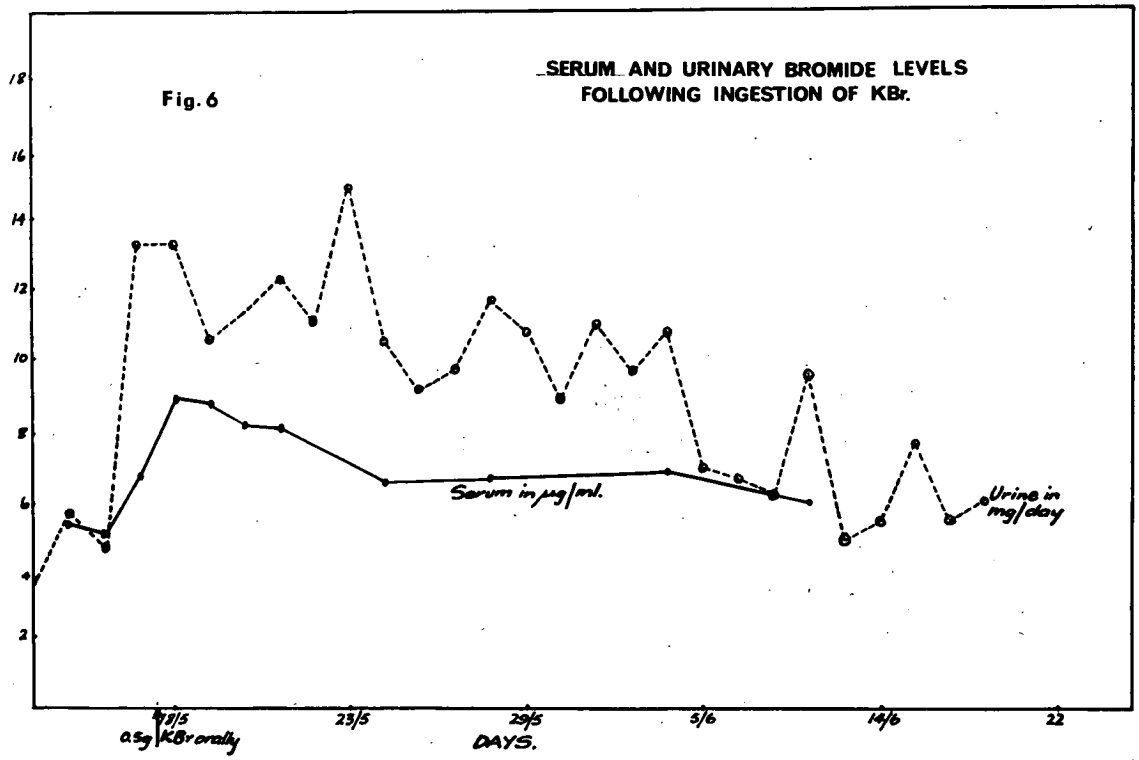
TABLE 17

Date	Urine			Serum
	Volume ml/24 hrs.	µg Br/ml	mg Br/24 hrs.	µg Br/ml
11.5.65	950	3.8	3.6	
12.5.65	990	5.7	5.6	4.3
13.5.65	1,330	3.5	4.7	4.0
*17.5.65	2,230	6.0	13.4	6.7
18.5.65	1,400	9.6	13.4	8.9
19.5.65	1,180	9.0	10.6	8.8
20.5.65	1,300	8.8	11.4	8.2
21.5.65	1,220	10.2	12.4	8.1
22.5.65	1,420	7.8	11.1	
23.5.65	1,570	10.0	15.0	
25.5.65	1,650	6.4	10.6	6.5
26.5.65	1,000	9.1	9.1	
27.5.65	1,250	7.8	9.8	
28.5.65	1,250	9.4	11.8	6.6
29.5.65	1,350	8.1	10.9	
30.5.65	1,400	6.3	8.8	
31.5.65	1,250	8.8	11.0	
2.6.65	1,000	9.7	9.7	
3.6.65	1,550	7.0	10.9	6.8
5.6.65	1,000	6.9	6.9	
6.6.65	900	7.3	6.6	
7.6.65	850	7.2	6.1	
11.6.65	1,500	6.4	9.6	5.9
13.6.65	800	6.0	4.8	
14.6.65	1,000	5.3	5.3	
15.6.65	1,350	5.6	7.6	
19.6.65	1,450	3.7	5.4	
20.6.65	1,600	3.7	5.9	

* Dose of 0.5 g K Br taken orally.

Fig. 6

SERUM AND URINARY BROMIDE LEVELS
FOLLOWING INGESTION OF KBr.



(b) The Effect of Bromide on Other Electrolytes.

Simultaneous investigations on the serum and urinary electrolytes following a dose of potassium bromide were carried out. The sodium and potassium content was measured on an Eel flame photometer and the chloride was measured by a mercuric nitrate titration method (146).

The results are shown in Table 18 and Figure 7.

(c) Diurnal Rhythm.

(1) In this experiment urine specimens were collected at six and eight hourly intervals for eight days from one human subject, and for three days from another. The volume of each individual specimen was measured and the urinary sodium, potassium, chloride and bromide contents were estimated. The results of these studies show the relative similarity of the diurnal excretory patterns of these ions, as seen in Tables 19a - d, and Figure 8. As both sets of results follow the same pattern, only one set is shown.

(2) The osmolality of each urine specimen was determined by a measurement of the freezing point depression. The depression of freezing point is determined by the number of osmotically active particles present per kg of solvent, and is linear within the range.

The urine osmolality is highest in the early morning specimen and lowest in the afternoon specimen. This is a reflection of the diurnal rhythm in the release of the anti-diuretic hormone from the posterior pituitary (147, 148). This hormone acts directly on the distal renal tubules to promote the reabsorption of water and consequently

TABLE 18

Date	Urinary Electrolytes				Serum Electrolytes			
	Urinary volume ml/24 hrs.	Na meq/ 24 hrs.	K meq/ 24 hrs.	Cl meq/ 24 hrs.	Na meq/l	K meq/l	Cl meq/l	Total protein g/100 ml
11.5.65	950	161.5	60.8	171	141	3.9	102	6.7
12.5.65	990	227.7	63.3	243.5	137	4.2	107	6.7
13.5.65	1330	199.5	85.1	215.4				
17.5.65	2230	205.1	62.4	200	137	3.8	102	6.6
18.5.65	1400	203	56	186.2	126	5.0	97	6.1
19.5.65	1180	165.2	61.3	174.6	137	4.0	104	7.1
20.5.65	1300	182	65	195	135	4.3	102	
21.5.65	1220	225.7	82.9	256.2	137	4.0	104	7.0
22.5.65	1420	241.4	71	249.9				
23.5.65	1570	251.2	72.2	259				
25.5.65	1650	184.8	66	199.6				

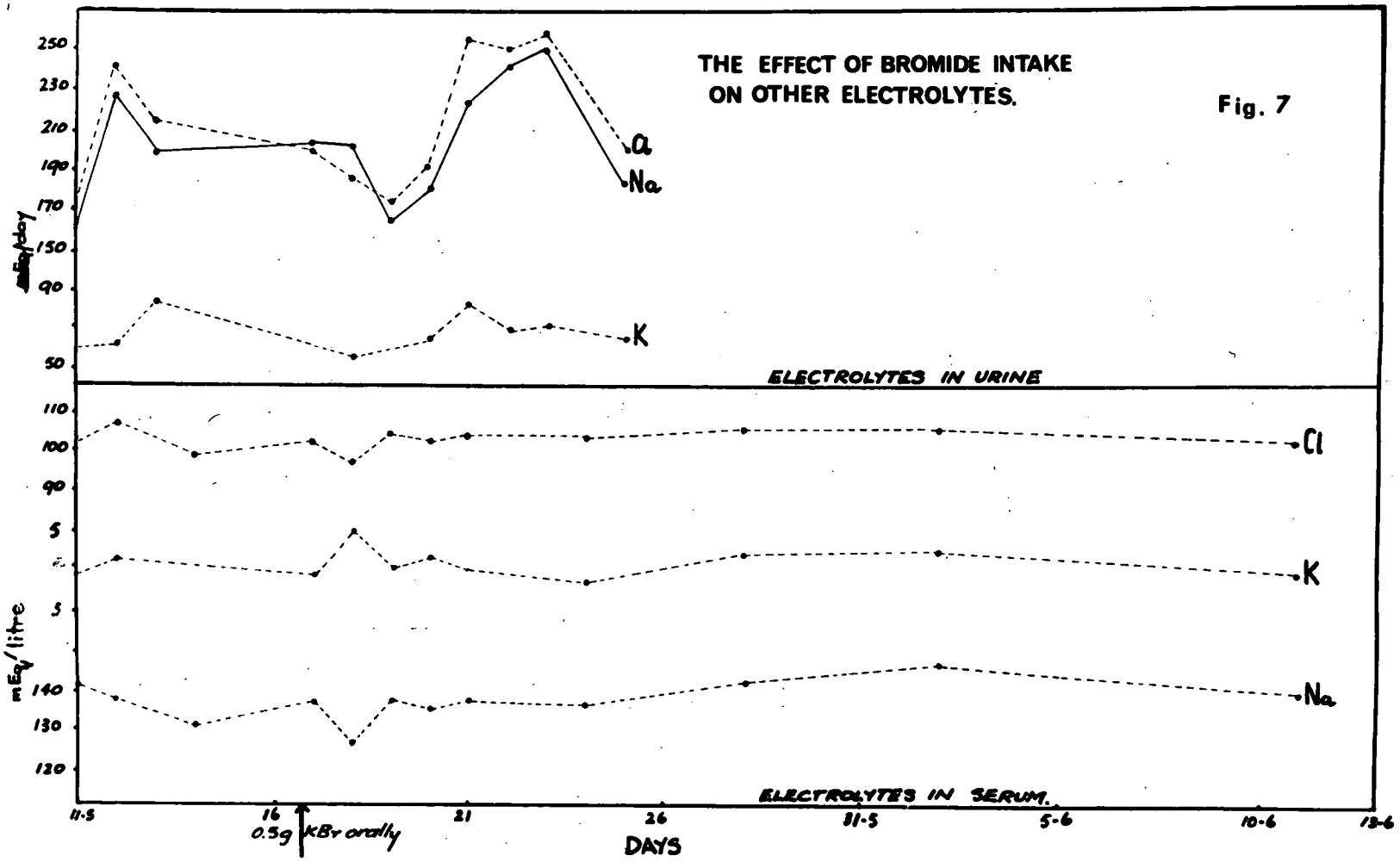

THE EFFECT OF BROMIDE INTAKE
ON OTHER ELECTROLYTES.
Fig. 7
250
230
210
190
170
150
90
50
mEq/day
Cl
Na
K
ELECTROLYTES IN URINE
110
100
90
5
5
140
130
120
mEq/litre
Cl
K
Na
ELECTROLYTES IN SERUM.
11-5
16
0.5g KBr orally
21
26
31-5
5-6
10-6
13-6
DAYS

TABLE 19a

DIURNAL RHYTHM

Day	1					2			
Period of day	12 MN ↓ 6 AM	6 AM ↓ 12 MD	12 MD ↓ 6 PM	6 PM ↓ 12 MN	Total / 24 hr.	12 MN ↓ 8 AM	8 AM ↓ 4 PM	4 PM ↓ 12 MN	Total / 24 hr.
Urinary volume /ml	160	260	290	400	1,110	600	420	880	1,900
mg Br/ specimen	0.42	1.30	1.07	1.00	3.79	1.14	2.10	1.32	4.56
m-equiv Cl/ specimen	16.8	48.8	49.2	41.2	156	54.4	84.0	65.5	203.9
m-equiv Na^+/ specimen	17.6	51.9	44.9	41.2	155.6	55.6	94.5	70.1	220.2
m-equiv K^+/ specimen	10.2	22.3	19.1	17.6	69.2	34.7	34.4	22.8	91.8
Osmolality	958	1064	968	583		593	859	355	

TABLE 19b

DIURNAL RHYTHM

Day	3				4				
Period of day	12 MN ↓ 8 AM	8 AM ↓ 4 AM	4 PM ↓ 12 MN	Total / 24 hr.	12 MN ↓ 6 AM	6 AM ↓ 12 MD	12 MD ↓ 6 PM	6 PM ↓ 12 MN	Total / 24 hr.
Urinary volume /ml	390	420	860	1,670	320	360	190	190	1,060
mg Br/ specimen	1.25	1.80	1.98	5.03	0.32	0.94	0.70	0.55	2.51
m-equiv Cl/ specimen	57.8	81.5	100	239.3	19.8	50.0	33.7	30.0	133.5
m-equiv Na^+/ specimen	62.5	92.4	116.3	271.2	23.3	52.2	38.0	38.0	151.5
m-equiv K^+/ specimen	23.4	30.2	25.9	79.5	9.6	19.4	14.1	19.1	62.2
Osmolality	748	801	570		443	712	829	844	

TABLE 19c

DIURNAL RHYTHM

Day	5				6			
Period of day	12 MN ↓ 8 AM	8 AM ↓ 4 PM	4 PM ↓ 12 MN	Total / 24 hr.	12 MN ↓ 8 AM	8 AM ↓ 4 PM	4 PM ↓ 12 MN	Total / 24 hr.
Urinary volume / ml	180	400	470	1,050	300	450	620	1,370
mg Br/ specimen	0.45	1.24	1.27	2.96	0.75	2.12	1.24	4.11
m-equiv Cl/ specimen	25.2	62.0	64.6	151.8	31.5	91.4	50.3	173.2
m-equiv Na^+/ specimen	26.1	62.8	74.1	163	30.0	85.6	49.7	165.3
m-equiv K^+/ specimen	15.5	26.4	19.8	61.7	15.0	32.4	19.9	67.3
Osmolality	9.66	794	733		700	859	436	

TABLE 19d

DIURNAL RHYTHM

Day	7				8				
Period of day	12 MN ↓ 8 AM	8 AM ↓ 4 PM	4 PM ↓ 12 MN	Total / 24 hr.	12 MN ↓ 6 AM	6 AM ↓ 12 MD	12 MD ↓ 6 PM	6 PM ↓ 12 MN	Total / 24 hr.
Urinary volume /ml	460	400	350	1,210	330	350	630	250	1,560
mg Br/ specimen.	1.20	2.0	1.58	4.78	0.66	1.23	2.21	1.15	5.24
m-equiv Cl/ specimen	44.2	72.4	64.7	181.3	24.1	48.3	82.3	47.8	202.5
m-equiv Na^+/ specimen	47.0	84.0	76.9	207.9	27.7	48.9	74.7	50.0	201.3
m-equiv K^+/ specimen	25.8	36.8	23.1	85.7	13.9	20.3	31.6	13.5	79.3
Osmolality	587	835	871		548	632	484	748	

DIURNAL RHYTHM OF ELECTROLYTE EXCRETION

Fig. 8

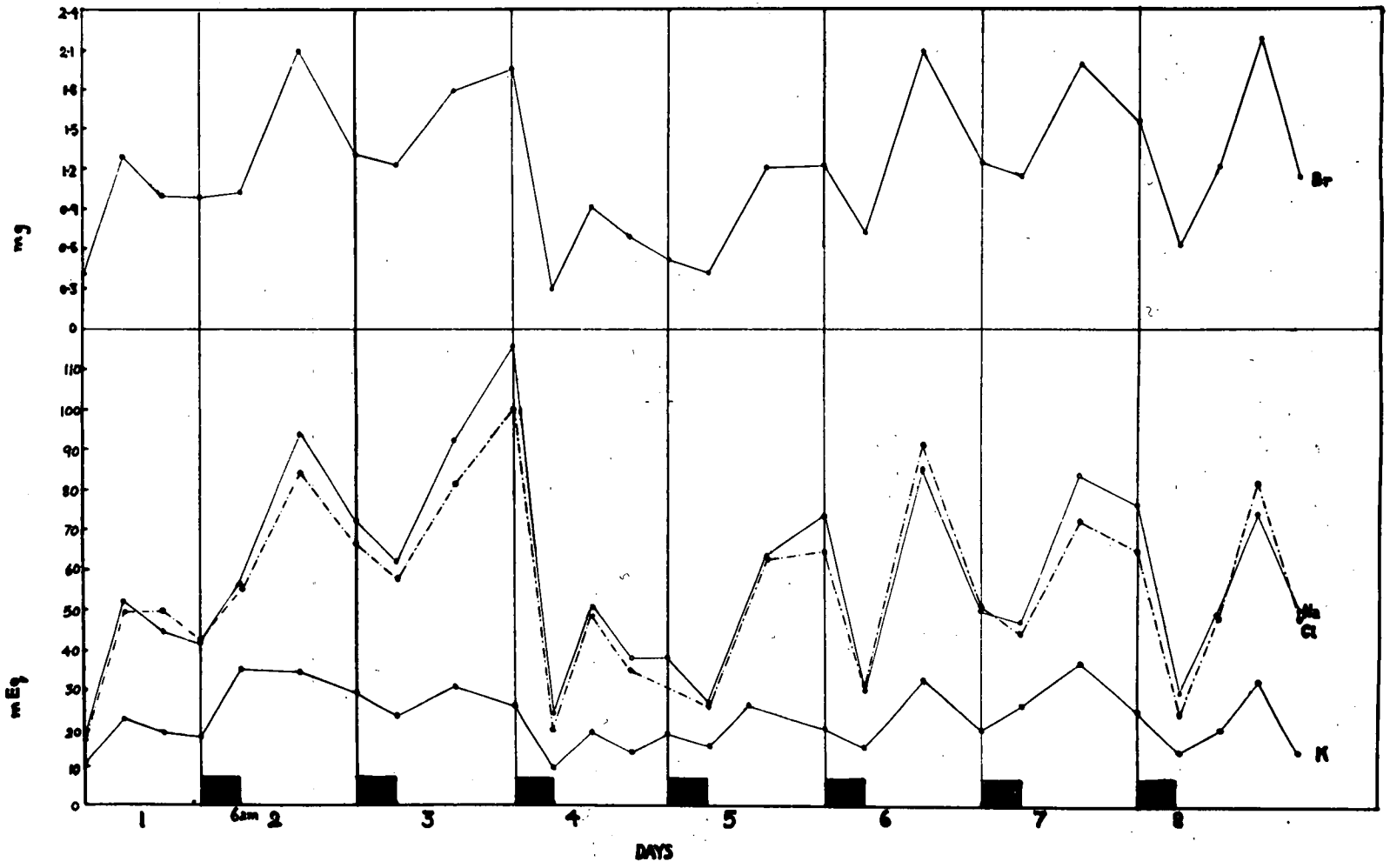
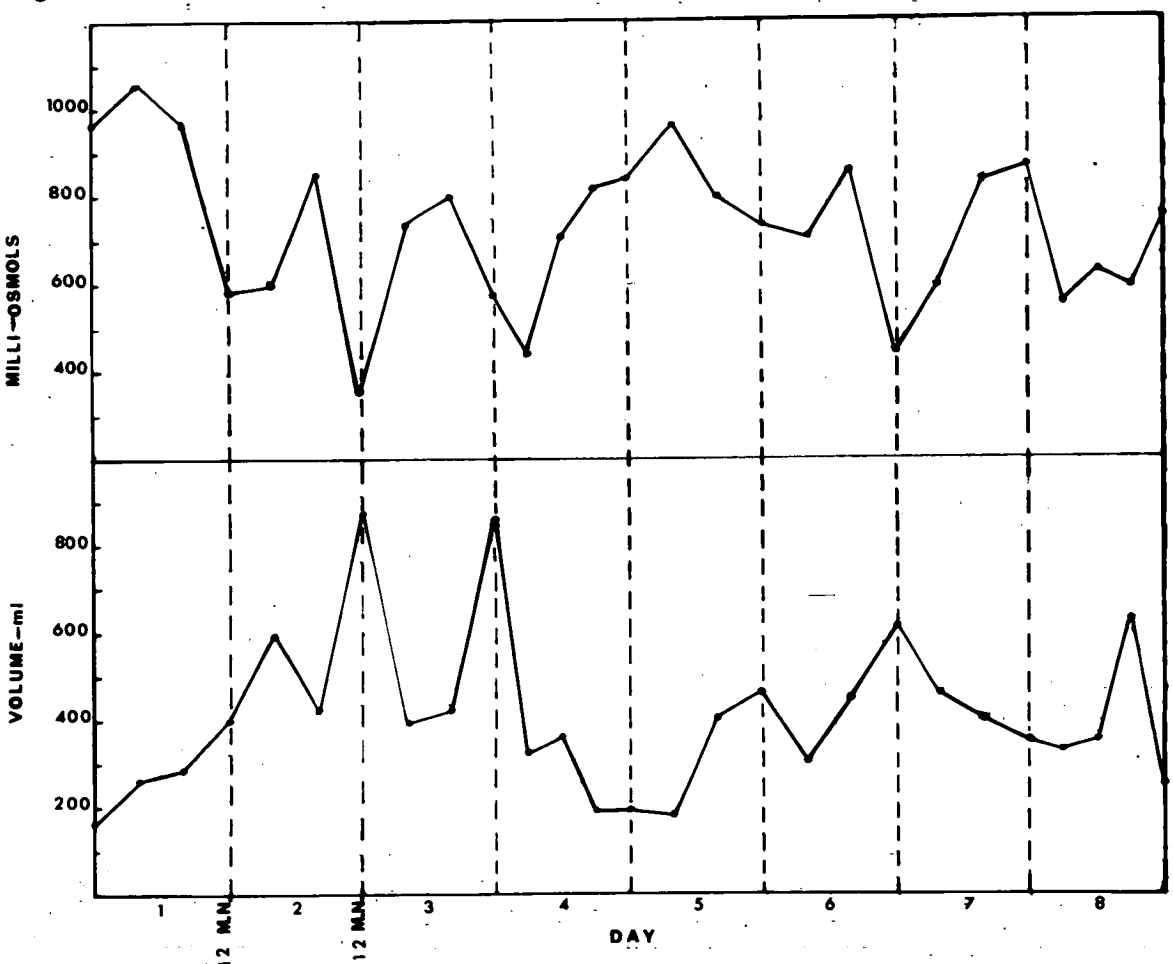


Fig. 9 DIURNAL RHYTHM OF URINARY VOLUME AND OSMOLALITY



there is a reciprocal relationship between urinary volume and concentration. The relationship between urinary volume and osmolality is shown in Figure 9.

DISCUSSION.

(a) Excretion Rate.

It can be seen from the results of the retention test that any significant excretion of Br^{82} occurred only after the first 24 hours following administration, as during this period only 2 - 3% of Br^{82} was excreted from the body. The average time of 50% retention, i.e. the average biological half-life in 4 human subjects, was 140 hours (6 days) which is lower than the figure of 8 days quoted in "The Recommendation of the I.C.R.P. - Permissible Dose for Internal Radiation", p. 174 (1959) and also that of Söremark (123) who claimed that it was 12 days.

These results differed from those of Cheek (149) and Forbes et al. (150) who reported that after $2\frac{1}{2}$ hours the distribution of bromide reaches a stable level and at the most increases by 2% from 3 to 24 hours and thereafter may remain constant for several days.

The 6 day bromide biological half life was also found to be the case following the oral intake of 0.5 g potassium bromide, as shown in Figure 6. This bromide estimation, which was made by chemical means, also indicated that although the serum and urinary bromide levels increased after the dose of K Br, the serum bromide level dropped by 50% in about one week, while the urinary bromide level which was subject to

considerable variation by comparison took about 2 weeks to drop to the 50% level and did not reach normal values until 4 weeks had elapsed. These findings support the claim of Palmer and Clarke (23) that bromide excretion is not always dependent on the serum bromide level.

The R urine/R serum bromide ratio was also calculated from available data which covered a period of 8 days, where R is the ratio of bromide to bromide plus chloride. The results are shown in the following table:

R urine/R serum ratio (Ru/Rs)

$$\text{where R urine} = \frac{\text{m-eq Br/l urine}}{(\text{m-eq Br/l urine} + \text{m-eq Cl/l urine})}$$

$$\text{and R serum} = \frac{\text{m-eq Br/l serum}}{(\text{m-eq Br/l serum} + \text{m-eq Cl/l serum})}$$

A single dose of K Br 0.5 g was taken orally.

Date	$\frac{Br}{Br + Cl}$ Serum	$\frac{Br}{Br + Cl}$ Urine	Ru/Rs
4 days before dose:			
12.5.65	0.000504	0.000288	0.57
Day of dose:			
17.5.65	0.000822	0.000832	1.01
18.5.65	0.001143	0.000901	0.79
19.5.65	0.001056	0.000756	0.72
20.5.65	0.000999	0.000733	0.73
21.5.65	0.000979	0.000604	0.62

In considering the Ru/Rs ratio it is necessary to point out that before the intake of 0.5 g K Br this figure was 0.57 (see above table). During the first 24 hours following the dose of K Br the ratio rose to 1.01, then on the second day it dropped to 0.79, while on the fourth day it was 0.62. Therefore, the Ru/Rs ratio reached unity only within the first 24 hours. At first sight it may appear that this sudden increase was due to a greatly increased urinary output of 2,230 ml/24 hours, as compared with an average of approximately half that amount on the preceding 3 days (see Table 17). Closer examination reveals that, although the urinary output returned to its previous average amount on the subsequent four days, the urinary bromide level remained high throughout this period. There must therefore be another reason for the Ru/Rs ratio reaching unity during the first 24 hours after the dose. If this 1.01 ratio is compared with one of 0.79 obtained the following day, some reasons for this difference can be seen.

Firstly, before the administration of bromide the average daily bromide excretion was approximately 4.6 mg. The dose taken was 0.5 g K Br, which represents 335 mg of bromide, and during the first 24 hours 13.4 mg of bromide was excreted which was 8.8 mg (13.4 - 4.6 mg) above the normal daily excretion. This represents approximately 2% of the total amount of K Br. In the retention experiment on 4 human subjects it was shown that during the first 24 hours only 2 - 3% is excreted; therefore the findings of both experiments are in agreement.

However it is known (see Chapter I) that it takes approximately 24 hours after the intake of bromide for equilibrium to be established.

During the first day after intake the serum bromide level was 6.7 μg/ml, and on the second and subsequent days 8.9 μg/ml. It is this lower serum bromide level during the first 24 hours which affects the $\frac{Br}{Br + Cl}$ ratio in the serum, as an increased amount of bromide is excreted in the urine regardless of the serum level, and thus the Ru/Rs ratio increases to unity. On the second day the bromide becomes more evenly distributed throughout the body; therefore the serum bromide level increases while the urinary bromide is almost unchanged, and thus the R serum increases and the Ru/Rs ratio is decreased to 0.79.

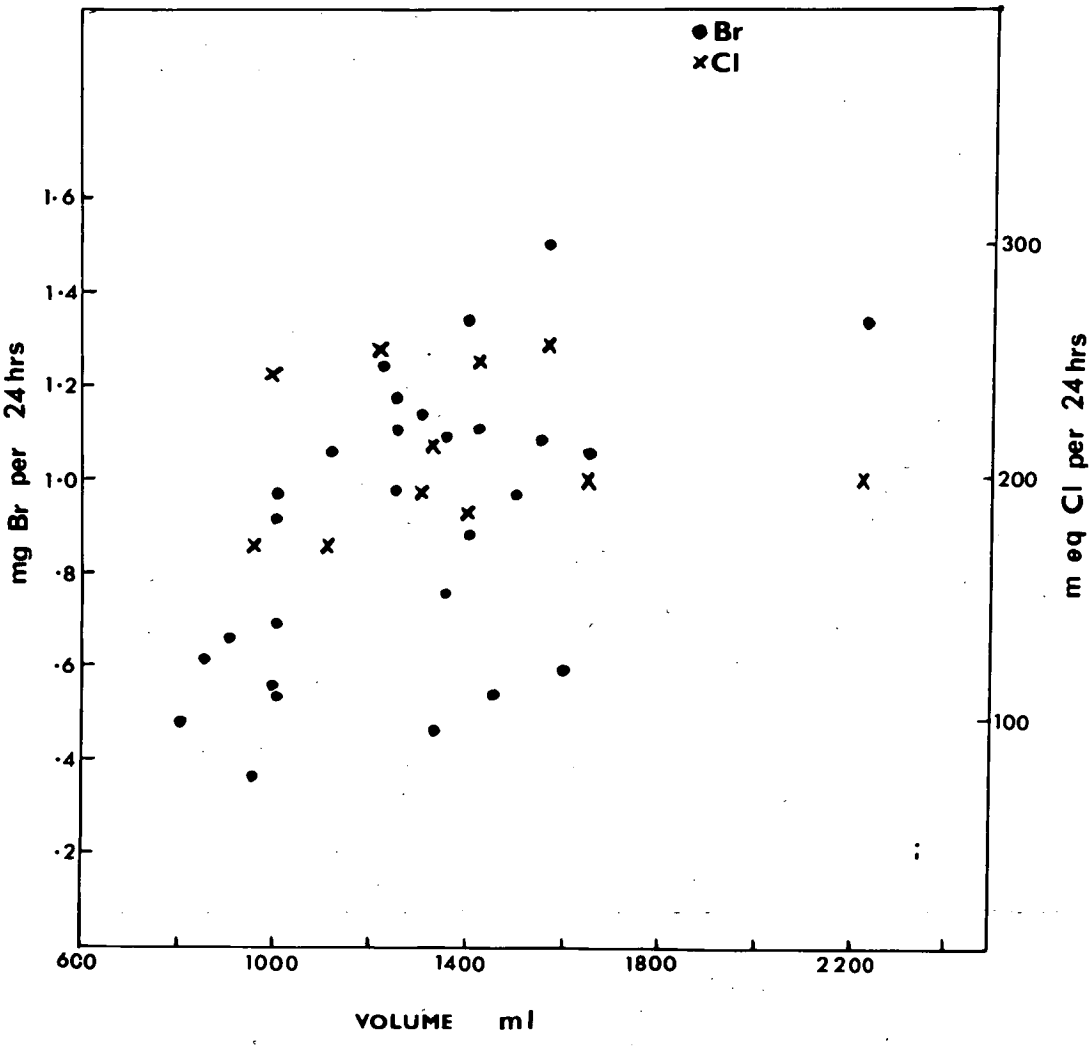
The Ru/Rs ratio was also performed on three human controls with normal serum bromide levels with the resultant figures: 0.42, 0.62, 0.68, all well below unity. These results and also the figures seen in the above table support the findings of other workers who reported that bromide is not excreted by the kidney at the same rate as chloride (23, 24, 71).

As has been mentioned, the increased urinary volume was not alone responsible for the increase in bromide excretion and the Ru/Rs ratio of unity. The relationship between the urinary volume and the concentration of bromide and of other electrolytes excreted was also investigated and is shown in Figures 10 and 11. The correlation coefficient (r) for urinary volume (x) and urinary bromide concentration (y) was calculated according to the formula:

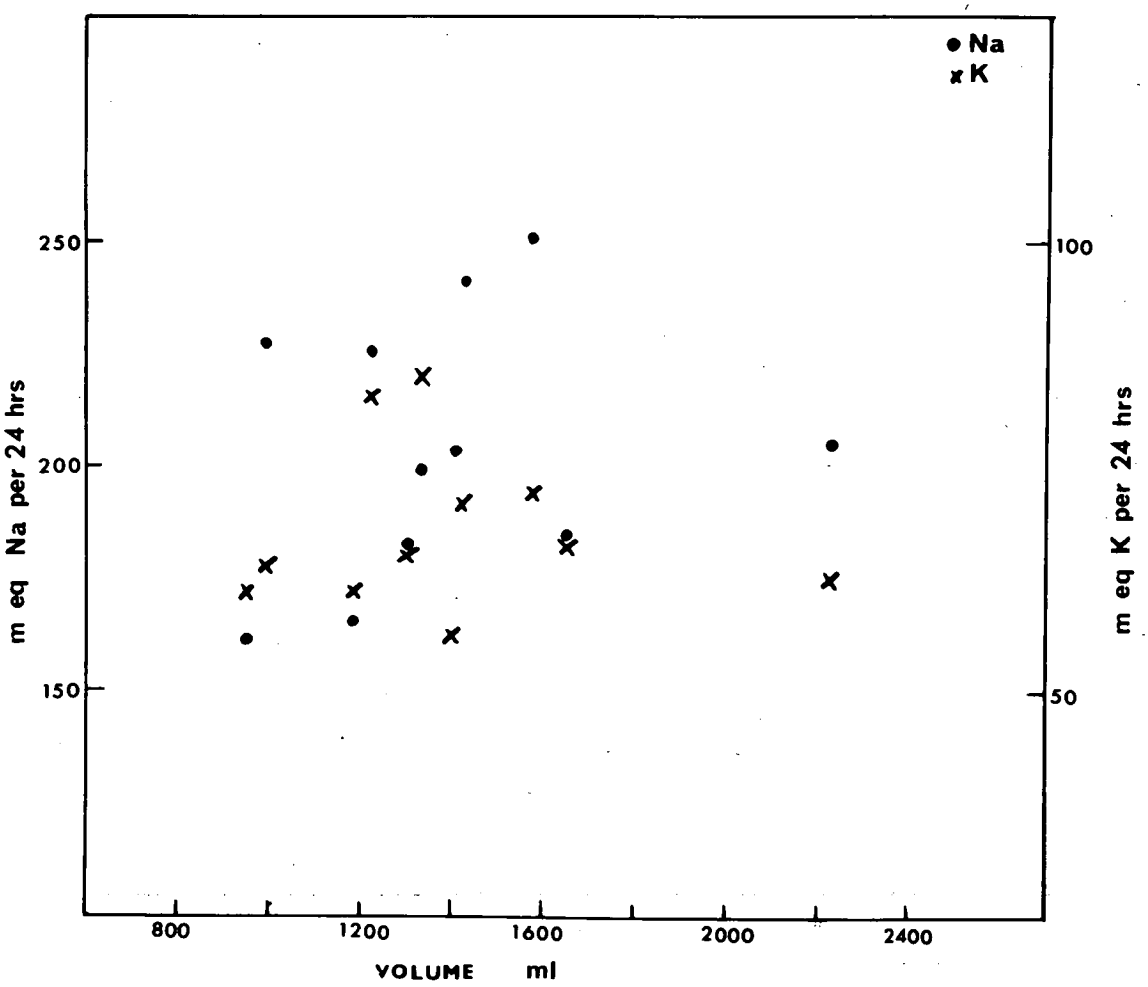
$$r = \frac{\frac{1}{N} \sum(x - \bar{x})(y - \bar{y})}{\sigma x \, \sigma y}$$

where $\bar{x}$ = mean of all x values, $\bar{y}$ = mean of all y values; σx = standard deviation of all x values and σy = standard deviation of all y values, and N = number of observations. The result of r = 0.60 and this did not indicate a high degree of correlation.

RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF BROMIDE AND CHLORIDE Fig. 10



RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF SODIUM AND POTASSIUM Fig. 11



(b) An investigation of the serum and urinary electrolytes following this single dose of K Br showed, (see Figure 7) that in the serum there was a drop in the levels of sodium and chloride, and a rise in that of potassium during the first 24 hours, which levelled off to normal in 48 hours. In the urine there was an increase in the levels of sodium and chloride and to a lesser extent of potassium, but this was not observed until 3 - 4 days after the dose of K Br.

(c) The diurnal rhythm of bromide excretion follows very closely that of sodium and chloride, as is shown in Figure 8. Table 19a - d indicates that the bromide content in the early morning specimens (i.e. from 12 m.n. to 6 - 8 a.m.) was lowest on all 8 days when compared with specimens collected during the day and in the evening. This was true regardless of the urinary volume of the early morning specimens.

The relationship between the urinary volume and electrolyte (including Br) excretion was also studied. The relationship between individual volumes and the bromide and chloride excretion in individual specimens for each of 8 days is seen in Figure 12, and a similar relationship for sodium and potassium in Figure 13. The total urinary volume per day was compared with the total excretion of bromide and chloride per day also. This is shown in Figure 14, and for sodium and potassium in Figure 15. The correlation coefficient (r) for the total daily urinary volume and urinary bromide concentration was calculated. This parameter was also estimated for sodium, potassium and chloride for comparison.

The results of r were: Br^- = 0.73

Cl^- = 0.85

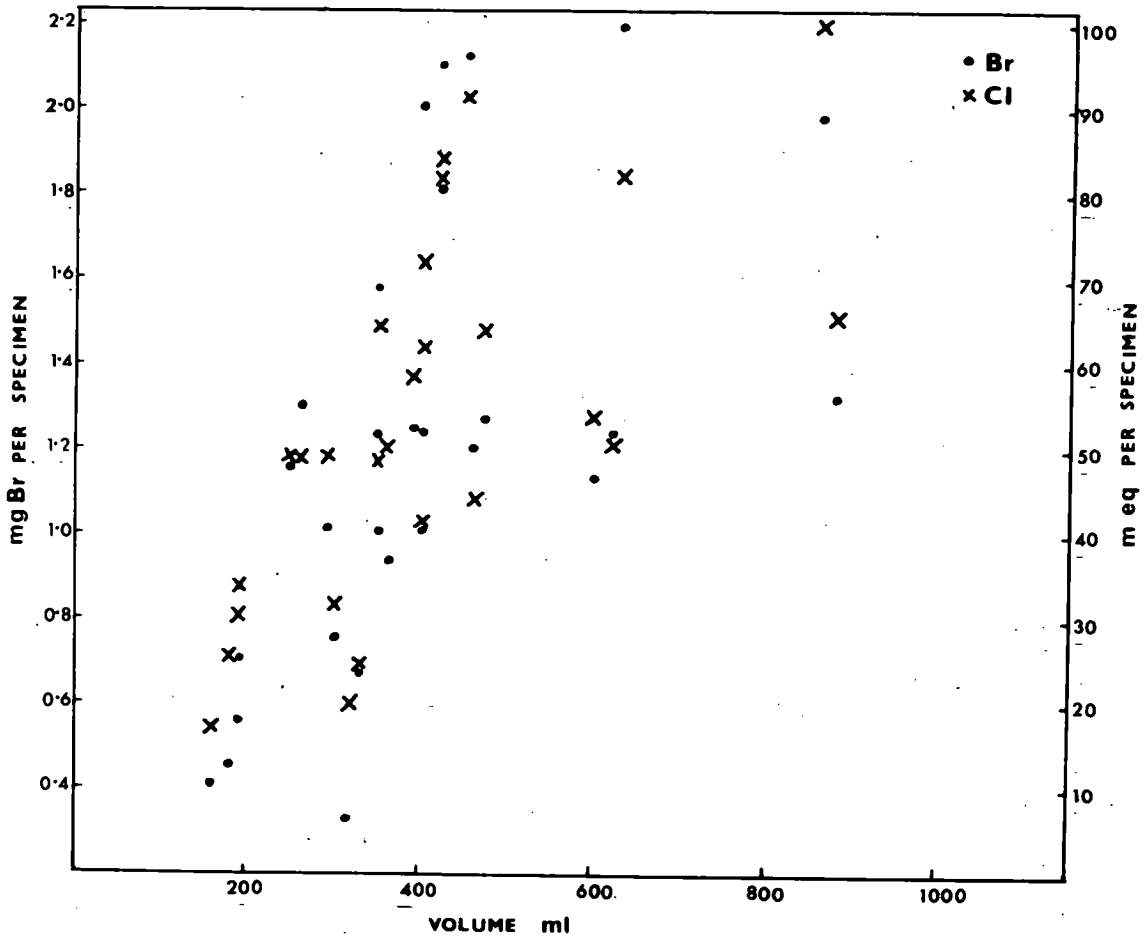
Na^+ = 0.76

K^+ = 0.78

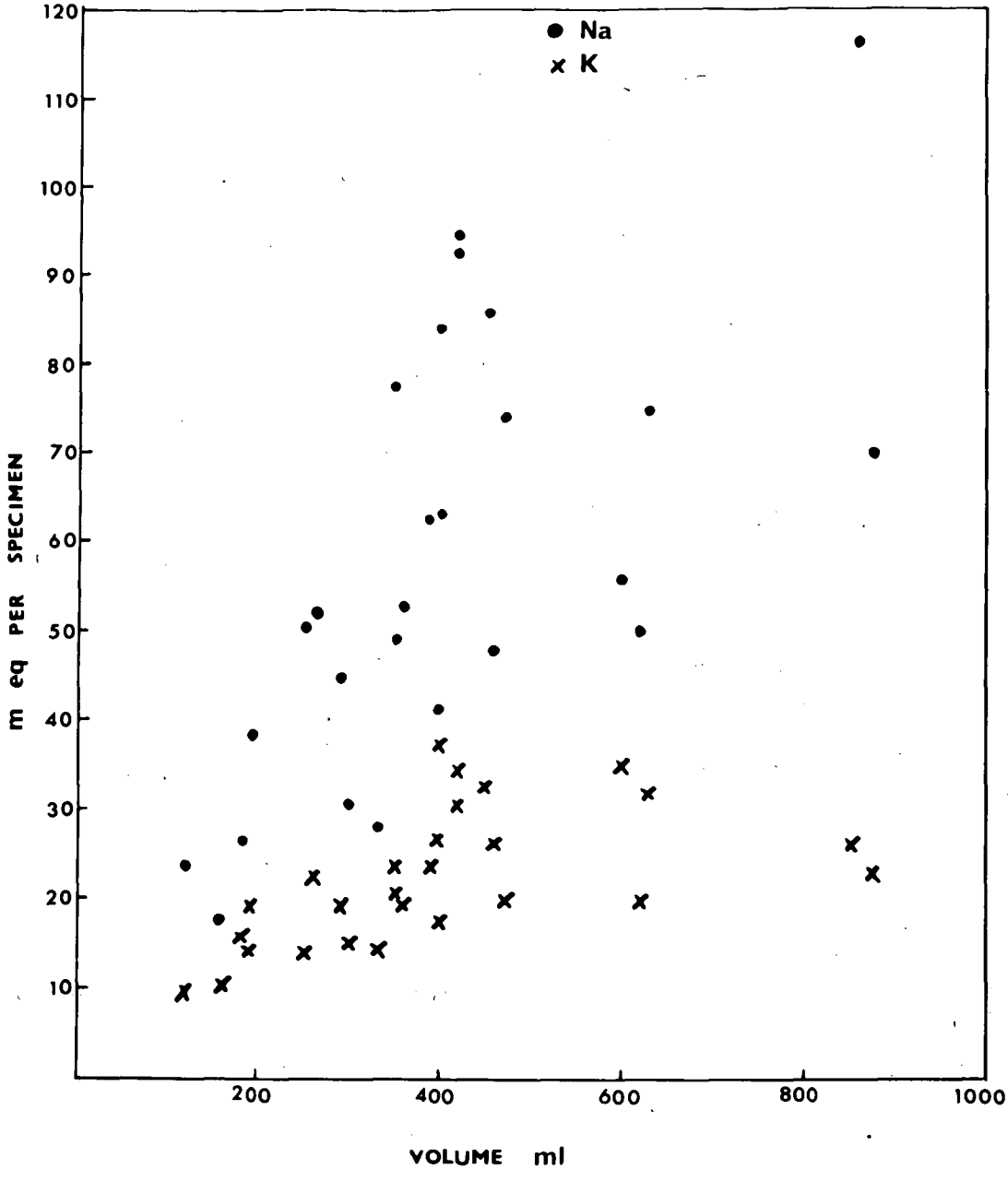
These results do not indicate a high degree of correlation between the urinary volume and the concentration of the above ions excreted in 24 hours.

RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF BROMIDE AND CHLORIDE .

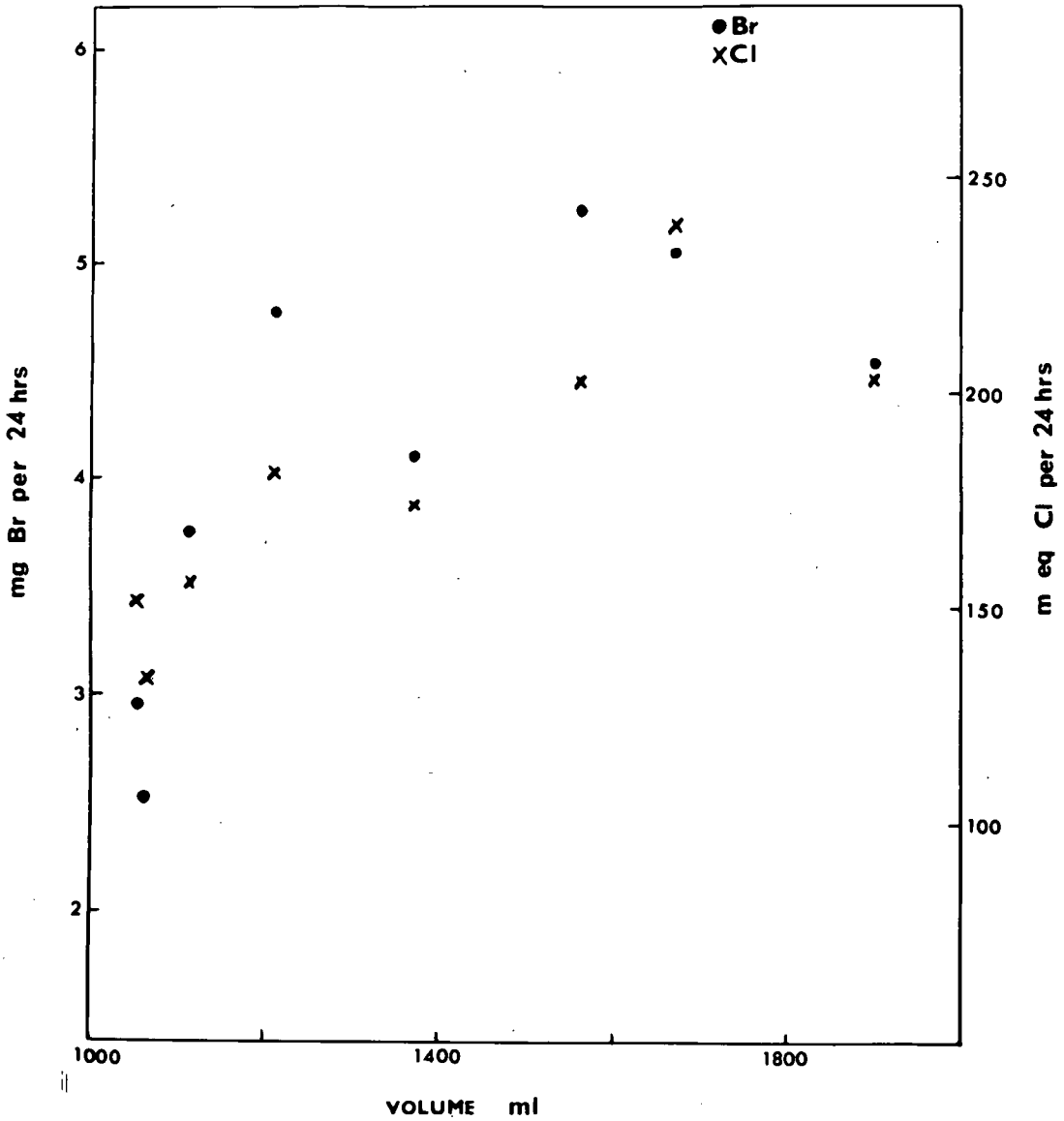
Fig. 12



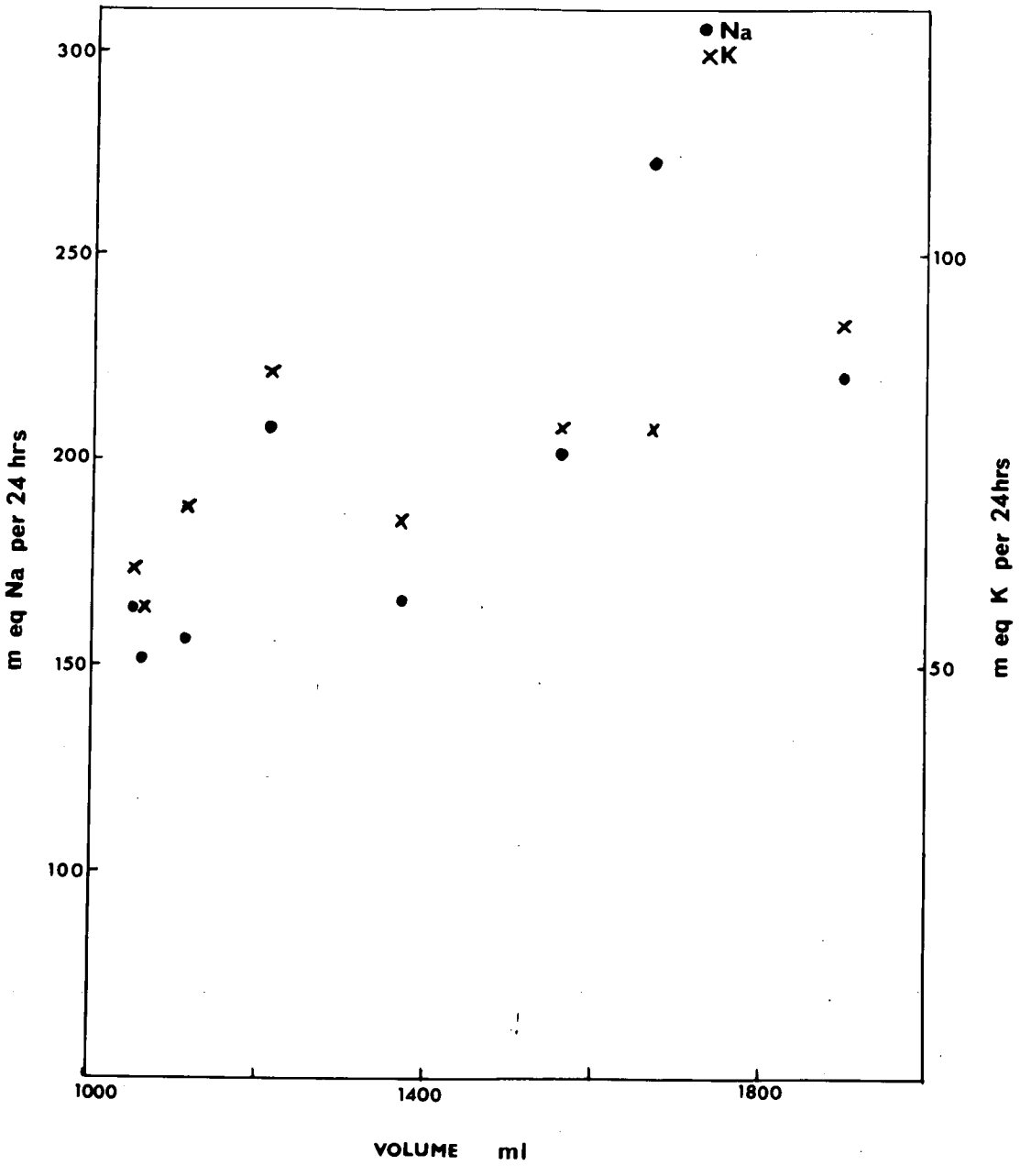
RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF SODIUM AND POTASSIUM Fig 13



RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF BROMIDE AND CHLORIDE Fig. 14



RELATION BETWEEN URINARY VOLUME AND CONCENTRATION OF SODIUM AND POTASSIUM Fig.15



CHAPTER V

PHARMACOLOGY AND TOXICOLOGY OF BROMIDE

PHARMACOLOGY.

Inorganic bromide is readily soluble in water and is rapidly absorbed into the bloodstream from the intestine (17). After absorption it is distributed throughout the body in almost precisely the same way as chloride, i.e. mainly in the extracellular fluid. It is generally assumed that bromide does not penetrate the cell membrane of most tissues except perhaps that of the thyroid and the red blood cells. However, Brattgard and Lindgvist (1954) (151) who studied the distribution of radioactive bromide in cats reported that bromide does occur in the cytoplasm and nucleus of the nerve cell.

THE MODE OF ACTION OF BROMIDE.

Bromide produces depressed activity of the central nervous system. This specific action is produced solely by the bromide ion and not by bromine in other forms such as bromates or organic compounds (17). Organically-bound bromine is a constituent of certain sedative drugs; however, there is strong evidence indicating that these drugs liberate bromide ions during their metabolism. The bromide ions bring about the depressing effect on the central nervous system by acting directly on the nerve cell. The exact mechanism of this action is

unknown. Harris and Derian (1949) (152) have suggested that bromides produce their action by interfering with the formation of co-enzymes I and II, and that this action can be abolished by the administration of massive doses of nicotinic acid (this vitamin is necessary for the formation of co-enzymes I and II). This hypothesis is supported by their experimental findings of an increase in urinary excretion of porphyrins in dogs deficient in nicotinic acid, and also in dogs maintained at a blood bromide level of 300 mg/100 ml. There was, however, a complete absence of toxic manifestations in a third group of dogs with a serum bromide level of 300 mg/100 ml but on a diet supplemented with nicotinic acid (152).

Six patients with high blood bromide levels were also studied, and in all cases the excretion of coproporphyrin was found to have increased above normal levels. Two of the patients were treated with niacinamide in conjunction with sodium chloride therapy and the remaining four patients were given niacinamide only, while maintaining a relatively high serum bromide level. In both groups, clearance of symptoms was rapid with an average time of five days; one patient was completely free of symptoms with a serum bromide level of 275 mg/100 ml after six days of niacinamide administration (152). However, Evans (1955) (17) pointed out that more evidence is needed to substantiate this hypothesis.

Inorganic bromides have long been used for sedative purposes, and although their widespread use appears to have declined, there are still several organic compounds containing bromine in common use as sedative and hypnotic drugs. The most notable of these are the two

bromo-ureides, carbromal (bromo-diethylacetylurea) and bromvaletone (bromo-dimethylpropylurea). Several proprietary lines of sleeping tablets, including "Relaxa-Tabs", contain one or both drugs (132).

TOXICOLOGY.

It has been shown that administered bromide acts as a sedative, while it is a known fact that the prolonged intake of large doses of bromide usually leads to bromide intoxication. According to Evans (17) bromide intoxication may be defined as a state of disordered mental and physical function in which there is (1) a raised serum bromide level, and (2) recovery when excess bromides are eliminated from the body. Acute bromide intoxication is said not to occur, as it is not possible to take a single poisonous dose of bromide without producing vomiting; hence the term, bromide intoxication, always implies a chronic condition.

Mental symptoms of bromide intoxication are, in the mildest cases, dullness, fatigue, difficulty in concentration and headache. When intoxication becomes more severe the disturbance in mental functioning reaches psychotic proportions, e.g. disorientation and hallucinations, and neurological symptoms such as slurred speech and tremor of the hands may also be present (17).

It was thought that there exists a threshold in the serum bromide level above which symptoms of bromide intoxication appear. However it has now been recognised that, although symptoms of bromide intoxication are usually associated with a high serum bromide level,

there is no uniform threshold at which symptoms appear. Barbour (153) reported one patient with a serum bromide level of 380 mg/100 ml (normal 0 - 2.0 mg/100 ml, according to different authors) without any clinical evidence of bromide intoxication. On the other hand, Campbell (154) observed severe symptoms with a serum bromide level of only 50 mg NaBr/100 ml. There are other authors who agree that there is no correlation between bromide intake and the severity of mental symptoms (155, 156, 157). Thus it is now recognised that there are individual variations in the physiological effect of bromide, and that the appearance of symptoms of bromide intoxication may be influenced by several factors such as age, the presence of vascular and renal disease, the duration of bromide intake, the intake of chloride, the excretion rate of bromide, the general state of health, and the emotional state.

Barbour (153) suggested that the simultaneous use of alcohol can lower the threshold considerably. Campbell (154) pointed out that chronic alcoholics are susceptible to bromide accumulation because of dehydration and a low blood chloride level. Pihkanen and Harenko (157) supported these findings.

The danger of inorganic bromide intoxication has been known for a considerable time. Nevertheless, the possible toxic effect of the indiscriminate use of organic bromine compounds is not widely realised. It has been thought by some that inorganic bromide is not readily freed from combination in the bromo-ureide molecule, and therefore is unlikely to cause a build-up of bromide, although as early as 1907 Eeckhout (158) suggested that bromvaletone could cause toxic

symptoms similar to those caused by inorganic bromides. Takeda (1911) (159) denied this and maintained that there was no danger of any toxic effects due to the bromide in the molecule because only a fraction of the bromvaletone was broken down in the body. He claimed that the hypnotic action of bromvaletone was due to unchanged ureide. Kwam (1912) (160) reported the same to be true for carbromal.

Magnussen (1947) (161) urged caution with the use of bromo-ureides because of their toxic effect. His experience and, since then, that of many others (122, 132, 162, 163, 164, 165, 166, 167, 168) who have treated cases of bromide intoxication due to the intake of bromo-ureides strongly supported the original suggestion of Eeckhout.

Shaw and Shaw (169) found a similar increase in the serum bromide level for a comparable daily intake of bromo-ureide and inorganic bromide. Trethowan and Pawloff (163) compared the rise of the serum bromide level in a group of human subjects who were on a daily intake of potassium bromide with a second group which was given a comparable amount of bromine in organic form, viz. Sedexin tablets. (Sedexin is a preparation identical to "Relaxa-Tabs" containing carbromal and bromvaletone in a 3 : 2 ratio.) They concluded from this experiment that, in the initial stages at least, bromo-ureide preparations produce a rise in the serum bromide level of a similar order to that obtained by the administration of an equivalent amount of inorganic bromide.

The toxic effect of the bromo-ureides has been found to be

associated with varying serum bromide levels, e.g. similar individual variations have been observed just as with the intake of inorganic bromide. Ronald (170) reported a case where carbromal intoxication occurred without any rise in the serum bromide level. Similar cases were reported by Kay and Copas (162) and Pihkanen and Harenko (157). Although Andrews (122) also had 19 patients with a known history of the intake of bromo-ureides who had a normal serum bromide level, nevertheless this author concluded from the results of a seven-year survey of 400 patients that amounts of bromo-ureides, well within the recommended dosage, if taken consistently for many months will produce high bromide levels in the serum. The toxic effect from bromo-ureides was more often associated with comparatively low serum bromide levels than has been the case with inorganic bromides (122). Similar results were associated with a serum level between 30 - 100 mg Br/100 ml (132, 157, 163). This could indicate that the ureide itself, or some derivative thereof, is toxic without its being metabolised in the body to yield inorganic bromide. However, as Kay and Copas (162) pointed out, not all patients taking inorganic bromide develop high blood bromide levels. It is to be expected therefore that patients metabolising bromo-ureides to inorganic bromide need not necessarily have a high blood bromide level before toxic manifestations occur.

The results of the survey by Andrews (122) on 400 patients

showed that in at least 148 of these the raised serum bromide level was found to be definitely connected with an intake of bromo-ureides, mainly "Relaxa-Tabs". However the result showed considerable individual variation in the effects associated with various dosage levels of bromo-ureides. Some patients appeared to accumulate bromide from the bromo-ureide molecule much faster than others; again the reasons for this were several, but one of these was found to be the simultaneous intake of other drugs including alcohol.

There appears to be some uncertainty whether the toxic action of carbromal and bromvaletone is different from that of inorganic bromide, although Sattler (171) maintained that there is a difference. However, Copas et al. (1959) (132) point out that it is difficult to distinguish between their effect on the nervous system and the classical picture of bromism. Nevertheless, there are two points of difference to be mentioned. Firstly, carbromal and bromvaletone appear to be toxic to the retina whereas this change has not been observed in epileptic patients who have been treated with considerably larger doses of inorganic bromide. Crawford (166) also reported that a man taking from 130 to 260 mg carbromal daily for five years was found to have reversible cataracts which were cleared in approximately eight weeks from the time his carbromal was discontinued. Secondly, the skin reaction produced by carbromal in susceptible subjects is unlike the eruption of bromism (172).

It seems that the ureide molecule itself, or some closely related organic metabolite, exerts a hypnotic effect. Although it is

usually considered that the toxicity of the ureide molecule itself is low, nevertheless, as the above evidence indicates, large doses could be responsible for serious symptoms.

Andrews (122) showed that some patients who had been on large doses of bromo-ureides showed the toxic symptoms which are characteristic of bromism. However, in some other cases it was not possible to be sure how much of the effect was due to the bromo-ureide molecule and how much to the bromide released from it. It could be concluded therefore that these organic bromide compounds probably act on the central nervous system in two ways, viz. due to the release of inorganic bromide from the organic molecule and the effect of the ureide molecule itself. The resulting toxicity may thus be due to both moieties, although released bromide is more toxic, mainly because it tends to accumulate in the body.

The treatment of bromide intoxication is to discontinue bromide medication and to administer sodium chloride 8 - 12 g per day by mouth together with 4 litres of water daily.

In recent years, many articles (163, 173, 174) have appeared in the medical literature of a number of countries drawing attention to the risk of bromide intoxication derived from continuous bromide medication in various forms. Most of the warnings have come from the medical staffs of mental hospitals. They stress that bromide intoxication is still quite common as bromide, especially in tablet form, is often prescribed by general practitioners, and in addition, since bromide preparations can be bought over the counter in any pharmacy, self medication with these substances is also quite common. However, as

Trethowan and Pawloff (163) point out, there are different opinions about the desirability of allowing this state of affairs to persist. Some feel that steps should be taken to prevent the occurrence of bromide intoxication by a restriction on the free sale of these preparations, while others are of the opinion that as bromides are relatively "harmless", undue restriction on their sale may cause those who wish to tranquillise themselves to resort to more pernicious preparations.

PRESENT INVESTIGATION

AIMS.

(a) To study the effect of bromide intoxication in the human.

(1) To discover whether the serum bromide level is associated with an increase in the urinary excretion of porphyrins in humans, as Harris and Derian (152) claimed in the case of dogs.and man.

(2) To investigate cases of bromide intoxication.

(b) To study the effects of bromide administration in guinea-pigs.

(1) To determine whether the relatively long administration of bromide (and fluoride) has any significant effect on the serum level of sodium, potassium and particularly chloride.

(2) To estimate the acetylcholinesterase level in the red blood cells of animals treated with bromide (and fluoride). This enzyme is present in several tissues, but mainly in erythrocytes and nerve cells. Acetylcholine, a mediator of nerve impulses, is hydrolysed by this enzyme to give choline and acetic acid; accordingly this enzyme is very important in the generation and propagation of nerve impulses. As has been stated bromide acts on nerve cells, and the question arises whether there is any relationship between a high bromide (and fluoride) content in the body and the level of the above enzyme in red cells.

(3) To estimate alanine amino transferase, previously called glutamic pyruvic transaminase (GPT), and serum protein fractions to find

out whether there is a possible effect of bromide intake on the liver.

It is a well known fact that diseased liver cells release large quantities of an enzyme (GPT) which turns α-ketoglutarate in the presence of alanine into pyruvate and glutamate, i.e.

$$\alpha\text{-ketoglutarate} + \text{alanine} \overset{\text{GPT}}{\rightleftharpoons} \text{pyruvate} + \text{glutamate}.$$

The value of the determination of serum GPT as an aid in the diagnosis of damaged hepatic cells has been well established.. The estimation of the GPT level in bromide and fluoride treated animals as compared with the level in untreated controls would give some indication whether the bromide and fluoride administered had any deleterious effect on liver tissue.

METHODS AND RESULTS.

(a) (1) Three human subjects with bromide intoxication were investigated. Their serum bromide levels were measured, and 24 hour urine specimens were collected from each for porphyrin estimation, on admission to hospital. In addition these same tests were carried out on subject C during NaCl therapy at intervals of 8 and 16 days from the commencement of treatment. The results of these tests are shown in Table 20.

In the case of patient B the serum bromide level dropped to 41.6 mg/100 ml in approximately 3 weeks of NaCl therapy. At this stage the bromide and chloride levels were measured in the urine and in the

TABLE 20

Porphyrin Excretion in 3 Humans with Bromide Intoxication

Subject	A	B	C		
			1. (On admission)	2. (8 days after NaCl therapy)	3. (16 days after NaCl therapy)
Sex	F	M	M		
Age	32	72	32		
Serum bromide level mg/100 ml (N = 0 - 2.0)	160	178	170	72	28
Total urinary volume ml/24 hrs.	4,125	1,620	1,050	1,450	1,275
Coproporphyrin mg excreted in 24 hrs. (N = 100-250 mg/24 hrs)	170	120	80	160	100
Uroporphyrin mg excreted in 24 hrs. (N = 0 - 60 mg/24 hrs)	Nil	Nil	35	24	12

serum. The purpose of these estimations was to determine the ratio R urine/R serum during NaCl therapy:

$$\text{where R urine} = \frac{\text{m-eq Br/l urine}}{(\text{m-eq Br/l urine} + \text{m-eq Cl/l urine})}$$

$$\text{and R serum} = \frac{\text{m-eq Br/l serum}}{(\text{m-eq Br/l serum} + \text{m-eq Cl/l serum})}$$

The figures for this were:

$$\text{R urine} = 0.0222$$

$$\text{R serum} = 0.0415$$

$$\text{therefore R urine/R serum} = 0.53.$$

(2) Bromide intoxication:

The serum bromide level was estimated by the chemical method in three patients showing signs of bromide intoxication, and each had a serum bromide level above 150 mg/100 ml. In each case the bromide intoxication was found to be due to the intake of organic bromine in the form of **Relaxa-Tabs** over periods of several months. These patients were treated by NaCl therapy and during a period of several weeks their serum bromide levels dropped to almost normal.

(b) Experiment on Guinea-pigs.

In this experiment guinea-pigs were divided into three main groups:

1st control group, i.e. no addition of bromide to drinking water.

2nd group : 400 p.p.m. of bromide added to drinking water in the form of KBr or NaBr.

3rd group : 5 p.p.m. of sodium fluoride added to drinking water.

The number of animals in each group varied from 8 to 20. Each group was divided into several sub-groups of usually two animals per cage. After approximately eight weeks under the above conditions the animals were bled by heart puncture for analysis of the blood components listed below.

(1) Serum electrolytes:

Most of the sodium and potassium estimations were performed on an Eel flame photometer and the chloride levels were estimated by the mercuric nitrate titration method of Schales and Schales (146). The remaining electrolyte levels and the total serum protein levels were measured on a Technicon Auto-analyser (175).

(2) Acetylcholinesterase:

An electrometric method for the determination of red blood cell cholinesterase activity was first described by Michel (1949) (176) and modified by Burman (1962) (177). It involves measuring the decrease of pH that occurs when acetylcholine is incubated with the enzyme in a standard buffer. Acetic acid is liberated from acetylcholine, and thus a fall in pH is produced. This drop in pH is linear with time for the first 30 minutes, the fall being proportional to the amount of enzyme present. The red blood cells containing cholinesterase are haemolysed in saponin solution to release the enzyme. The pH was measured on a Radiometer pH meter model 27 B, Design 2.

(3) Serum GPT estimation:

The colorimetric method of Reitman and Frankel (178), modified by Newfield (179), was used. This method is based on the great difference in absorption at 505 mu of an alkaline solution of the 2, 4-dinitrophenylhydrazone of ∝-ketoglutarate, as compared with that of pyruvate. With an increase in pyruvate and a concurrent decrease in ∝-ketoglutarate, the resulting increase in optical density is proportional to the pyruvate that is produced. Electrophoretic patterns of the serum proteins on one animal from both the control and bromide group were also obtained. Figure 16 shows that there is no difference between the two patterns. The results of electrolytes, cholinesterase and GPT estimation are shown in Table 21.

The serum bromide level was estimated in most guinea-pigs. In the bromide group it varied from 350 - 420 ug/ml; in the control group it was within the normal range of 4.5 - 11 ug/ml, and in the fluoride group it was slightly lower than in the control group (4.0 - 7.5 ug/ml).

DISCUSSION.

(a) (1) It can be seen from the figures presented above that the amounts of porphyrins excreted were well within the normal ranges. These results are at variance with the findings of increased porphyrin excretion in both dogs and man by Harris and Derian (152). However, in the present study only 3 cases were examined and the highest serum bromide level was

ELECTROPHORETIC PATTERNS OF GUINEA PIG SERA

Fig. 16

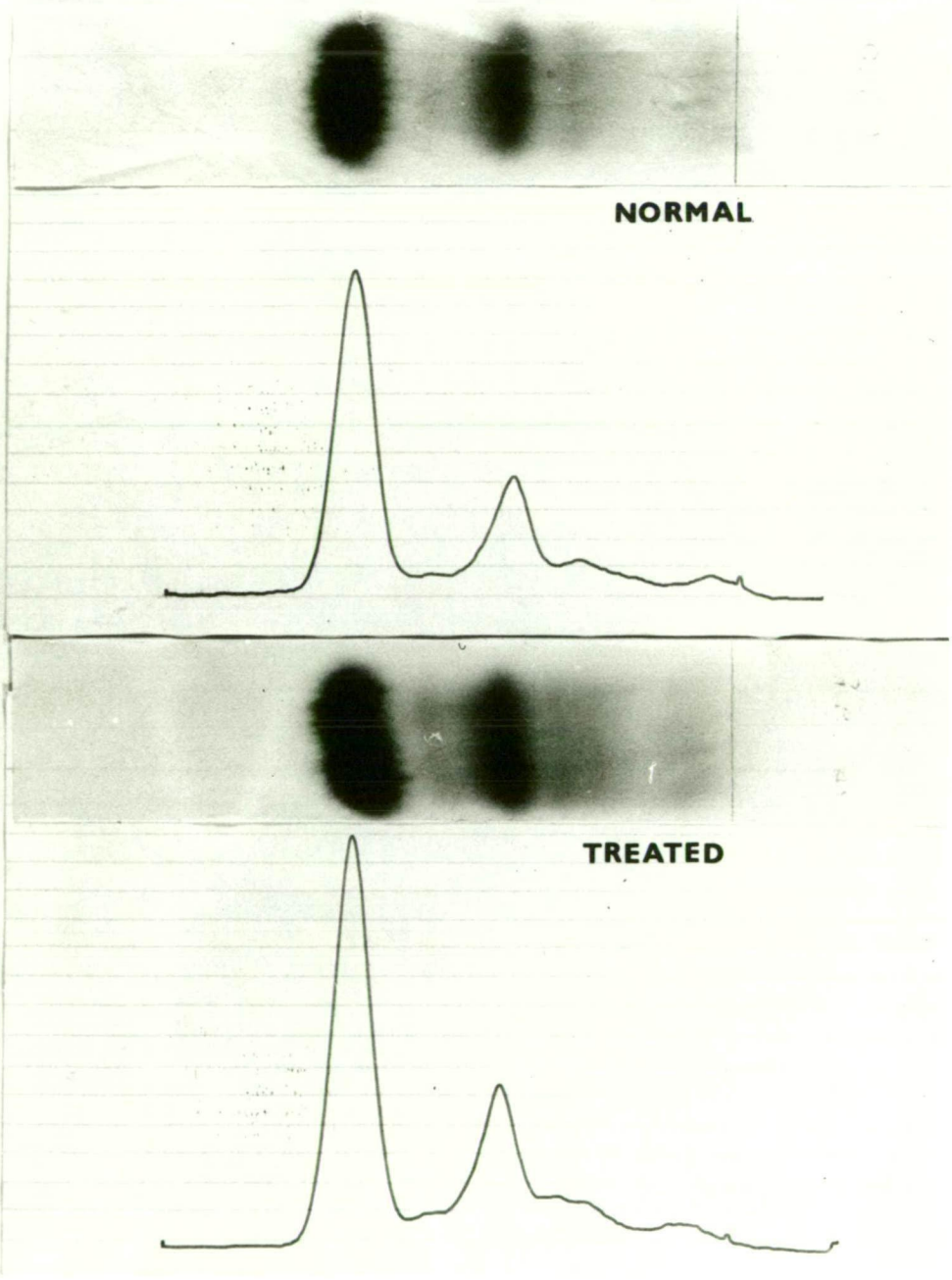


TABLE 21

THE INFLUENCE OF BROMIDE AND FLUORIDE INTAKE ON SOME BLOOD COMPONENTS

Control			400 ppm Br in drinking water (8 weeks)		5 ppm F in drinking water (8 weeks)	
Test	No. of specimens	Range	No. of specimens	Range	No. of specimens	Range
Na (m-eq/l)	20	132-146	9	132-150	8	133-155
K (m-eq/l)	12	5-7.2	5	4.4-7.9	4	5.3-7.6
Cl (m-eq/l)	20	97-116	8	102-122	6	100-120
Total protein (g/100 ml)	15	4.0-5.9	3	4.6-5.3	3	4.2-5.8
Cholinesterase (units/ml red blood cells)	11	1.3-2.6	11	1.3-2.8	5	1.0-2.2
G.P.T. (R and F units/ ml)	20	30-98	6	44-220	5	30-74

178 mg/100 ml. This level is significantly lower than the 300 mg/100 ml of the previously quoted authors, and this may account for the different results.

Another point worthy of mention in connection with the patient in question is the R urine/R serum ration, where R is the ratio of bromide to bromide plus chloride, i.e. $\frac{Br}{(Br + Cl)}$. Although the serum bromide level dropped from 178 mg/100 ml to 41.6 mg/100 ml during four weeks of NaCl therapy, R urine/R serum was still well below unity, i.e. 0.53. This occurred despite the considerable increase of total bromide excreted in the urine (i.e. 250 µg/ml as compared with the average 5.0 µg/ml in a normal human). This supports the view of Palmer and Clarke (23) and Evans (17) that NaCl therapy in bromide intoxication causes an increase in the total halide excreted; the absolute amount of bromide excreted is increased.

(2) Despite the fact that only 3 cases of bromide intoxication were analysed, these findings nevertheless indicate the danger of the indiscriminate use of various sedative drugs containing bromine. One patient had been taking one of these preparations for several months and was even carrying the tablets on admission to hospital.

(b) During the first two weeks of the experiment less water was drunk by the fluoride group and even much less again by the bromide group than the control animals. This presumably was due to the taste imparted to the water by the addition of fluoride and bromide. However, after a fortnight both groups drank approximately the same amount as the control

group. In the bromide group, there was no evidence of bromide intoxication beyond a slight sluggishness in the movements of the animals, while the fluoride group showed no effect at all.

After two months, the serum level in the bromide group of animals was 35 - 42 mg/100 ml, a relatively high level for guinea-pigs, but apparently not high enough to cause bromide intoxication.

(1) The serum electrolyte level in normal guinea-pigs, when compared with that of humans, shows that the sodium level is about the same, viz.: 132 - 146 m-eq/l in guinea-pigs as against 130 - 145 m-eq/l in humans. The potassium figures for guinea-pigs are slightly higher, viz.: 5 - 7.2 as against 3.5 - 5.5 m-eq/l, but since a certain small degree of haemolysis was unavoidably present in the specimens, this difference could be due to this factor. The chloride level, however, shows a pronounced difference as the analysis of specimens from 20 animals showed the range in guinea-pigs to be from 97 - 116 m-eq/l while the normal human range is 95 - 108 m-eq/l. The total serum protein level on the other hand is lower in guinea-pigs, with a range of 4.0 - 5.9 g/100 ml as compared with the human range of 6.2 - 7.6 g/100 ml.

The investigation of the individual fractions of serum proteins shows that there is not much difference in the albumin and the albumin-globulin ratio as seen from Table below. In this table the percentage of individual fractions of the total protein is given for normal humans, one normal guinea-pig and one guinea-pig on bromide. The table shows that, whereas the largest globulin fraction in humans is the γ-globulin, in guinea-pigs it appears to be α_2 component.

Comparison of Serum Protein Fractions in Humans and Guinea-pigs

(as % of Total Protein)

	Normal human	Normal guinea-pig	Guinea-pig from bromide group
Albumin	54 - 66	58	55
α_1 globulin	3 - 6	2	4
α_2 globulin	5 - 9	25	24
β globulin	9 - 15	10	11
γ globulin	11 - 22	5	6
Total serum globulin	31 - 52	42	45
Albumin/globulin ratio	1.2 - 2.0	1.38	1.22

In considering the effect of bromide and fluoride on the above electrolytes, the results obtained indicate that the intake of bromide and fluoride in the amounts given have no significant effect on the serum electrolytes and total serum protein level. Any small variations shown between the three groups could be attributed to the individual variations between the animals, and possible experimental error. It appears that the sodium and potassium ingested (since the bromide was given as the sodium and potassium salts) did not significantly alter the level of these ions in the serum and therefore most of the consumed sodium and potassium must have been excreted. The chloride levels were practically the same in all three groups, but as it is known that bromide can replace chloride from the tissue and blood, one would expect the chloride level in the bromide group to be lower than in the control

group. In actual fact, the figures for the bromide group are slightly higher. However it must be remembered that the serum bromide level of 35 - 42 mg/100 ml represents from 4.4 - 5.2 m-eq/l. The figures for the chloride level in the bromide group are on the average 5 m-eq/l higher compared with the control group. This can be simply explained by the fact that the mercuric nitrate titration method used for the chloride estimation does not differentiate between chloride and bromide, hence the extra bromide 5 m-eq/l in the bromide group has been measured as chloride, and hence the higher figures for chloride were obtained. Actually, the true serum chloride levels in both groups remain about equal. This shows, however, that at the above serum bromide level actual replacement of chloride is not significant.

(2) The level of acetylcholinesterase activity in normal guinea-pigs of from 1.3 - 2.6 units/ml of red blood cells is much lower than the normal human level of 6.8 - 12.2 units/ml of red blood cells. This perhaps would be expected in view of the higher development of the human central nervous system.

The level of acetylcholinesterase activities was almost identical in all three groups, which indicates that there is no direct effect of bromide or fluoride in the given doses on this enzyme. The slight decrease of the level of activity of this enzyme in the fluoride group may not be significant because only five samples were examined as against 11 in both the bromide and control groups.

(3) The serum GPT level in the 20 normal guinea-pigs examined showed a range of 30 - 98 R. and F. units/ml, which is higher than the

generally accepted range in normal human serum (5 - 35 R. and F. units/ml).

The dose of fluoride administered did not have any effect on the above enzyme, and it is probable that no effect on the GPT level could be attributed to the administration of bromide. The serum GPT level in the latter group ranged from 44 - 220 R. and F. units as against 30 - 98 in the control group but there were only two animals with serum GPT levels above the normal range. The actual figures in the bromide group were:

44, 58, 84, 85, 130 and 220 R. and F. units/ml.

The two highest levels could possibly be due to liver damage caused by some other factor than that of administered bromide. However the secondary and supplementary effect of bromide in these animals cannot be excluded, although conclusive evidence cannot be presented at this stage to show that bromide causes any damage to liver cells and thus liberates GPT into the serum.

The electrophoretic pattern of an animal from the bromide group was identical with that of a control animal. This would be further evidence that prolonged administration of bromide in the doses used does not affect the liver, which is a very important site of serum protein production.

Therefore it can be concluded that there is no evidence to suggest that bromide and fluoride administered in the doses given have any significant effect on the level of the serum electrolytes, the total serum protein or the two enzymes, acetylcholinesterase and alanine amino transferase.

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